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(57) Abstract

HCAR and MCAR protein molecules are human and mouse proteins (respectively) that serve as cellular receptors for adenoviruses of the serotypes 2 and 5 (subgroup C) and for the group B coxsackieviruses. The invention provides DNA molecules encoding these proteins or encoding functional derivatives thereof, host cells transformed with the DNA molecules and methods of producing the recombinant proteins or derivatives. These proteins, peptide fragments of these proteins which correspond to their extracellular domains, as well as other functional derivatives, in particular oligopeptides which bind virus are also provided. Isolated HCAR or MCAR proteins or fragments or variants thereof are used to prevent or treat virus infections. Expressing the above DNA in cells which lack these viral receptors renders the cells susceptible to transformation by adenoviral vectors carrying genes for gene therapy.

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CELLULAR RECEPTORS FOR SUBGROUP C ADENOVIRUSES AND GROUP B COXSACKIEVIRUSES

BACKGROUND OF THE INVENTION

Field of the Invention

The invention in the field of virology, molecular genetics and medicine relates to the HCAR and MCAR protein molecules, human and mouse proteins (respectively) that serve as common cellular receptors for adenoviruses of the serotypes 2 and 5 (subgroup C) and for the group B coxsackieviruses. The invention provides peptide fragments of these proteins which correspond to the extracellular domains and more discrete virus-binding peptides, as well as other functional derivatives, DNA molecules encoding these proteins, peptides and functional derivatives, and antibodies to the proteins. The DNA molecules are used for recombinant production of the proteins and peptides. The isolated proteins or fragments or variants thereof are used to prevent or treat virus infections. The invention also concerns expressing the DNA encoding these virus receptors in cells which lack them, rendering the cells susceptible to transformation by adenoviral vectors carrying genes for gene therapy.

Description of the Background Art

Viruses infect cells by first attaching to the cell surface. This requires specific interactions between molecules on the surface of the virus and receptor molecules on the susceptible cell. A number of virus-specific cellular receptor molecules have been identified, most of which have other known cellular functions. For example, human immunodeficiency virus (HIV-1) binds to CD4 molecules and to chemokine receptors, Epstein-Barr virus binds to the complement receptor protein CR2, human rhinoviruses bind to the cell adhesion molecule ICAM-1, rabies virus binds to the acetylcholine receptor, reoviruses bind to β-adrenergic receptors and herpes simplex virus appears to use fibroblast growth factor receptor as a binding site. For a thorough review of cellular receptors for animal viruses, see Wimmer, E. (ed.) *Cellular Receptors for Animal Viruses,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1994, which is hereby incorporated by reference in its entirety.

The expression of these virus binding proteins or receptors is a strong determinant of susceptibility to virus infection. For example, binding is required for virus uptake by the target cell, an event that may occur at the cell surface or within an acidified endosome after receptor-mediated endocytosis (White *et al.*, *Quant. Rev. Biophys. 16*: 151-195 (1983)). After internalization, the virion nucleic acid enters the cytoplasm and the viral replication process is initiated.

Adenoviruses (Ad) are DNA viruses, some members of which are human pathogens. In general, viruses of different families do not compete for binding to a common receptor. However, two serotypes of adenovirus, serotype 2 (Ad2) and serotype 5 (Ad5), as well as the RNA virus group known as group B coxsackieviruses (CVB) are human pathogens that do share a common receptor (Lonberg-Holm, K. *et al., Nature 259*:679-681 (1976)). Adenoviruses attach to a cellular receptor with the "knob" portion of their capsid fiber (Xia, D. *et al., Struct. 2*:1259-1270 (1994)). In contrast, the CVB are presumed to attach to cells through insertion of the receptor into the canyons on the virion surface (Muckelbauer, J.K. *et al., Curr. Biol. 3*:653-667 (1995)). Various cellular molecules have been identified that interact with the adenoviruses (Wickham T.J. *et al., Cell 73*:309-319 (1993)) and CVB (de Verdugo, U.R. *et al., J. Virol. 69*:6751-6757 (1995); Mapoles, J.E. *et al., J. Virol. 55*:550-566 (1985)), but a putative common receptor has not been identified and cloned prior to the present invention.

New interest in understanding how two different viruses use the same receptor molecule has been revived after the initial observation of a common putative receptor for that Ad2, Ad5 and the CVB twenty years ago (Lonberg-Holm *et al., supra*). Studies by investigators in Crowell's laboratory have advanced the understanding the biology of CVB receptors over the past 15 years. However, all attempts at biochemical isolation led to limited success at best. Antibodies against cellular receptor material and various partial isolations using conventional techniques were reported by Crowell and colleagues (Xu, R. *et al., Virus Res.*, 1995, 35:323-40; Bergelson, J.M. *et al., J. Virol.*, 1995,

69:1903-1906; Hsu, K.H. *et al., J. Virol.*, 1989, 63:3105-3108; Hsu, K.H. *et al., J. Virol.*,, 1988, 62:1647-1652; Mohanty, J.G. *et al., Virus Res.* 1993, 29:305-320; Crowell, R.L. *et al., J. Virol.*, 1986, 57:438-445; Mapoles J.E. *et al., J. Virol.*, 1985, 55:560-566; Krah, D.L. *et al., J. Virol.*, 1985, 53:867-870). Similar work by other groups was also reported (Stevenson, S.C. *et al., J. Virol.*, 1995, 69:2850-2857; Defer, C. *et al., J. Virol.*, 1990, 64:3661-3673). However, despite biological, serological and some biochemical characterization of cellular material which appeared to behave as a viral receptor, neither cloning nor definitive isolation and of these proteins had been achieved. Certainly, it was not possible to characterize the domain structure of these proteins or to identify which regions are useful for the utilities described herein. Prior to the present invention, the art was unable to purify sufficient quantities of receptor material to allow partial amino acid sequencing necessary for cloning of the genes for these receptor proteins. Earlier attempts by the present inventors' colleagues had failed in this regard. The cloning of two such proteins was achieved for the first time by the present inventors.

There is a recognized need in the art to understand the pathogenesis of the CVB group of viruses due to the severe diseases they cause, including myocarditis (Melnick, J.L. in: *Virology* (Fields B.N. *et al.*, eds.) Raven, New York, 1990, pp. 549-600). Expression of the cellular receptor obviously influences (a) which tissues are most susceptible to infection and (b) by what routes the virus spreads through the body.

More recently, interest in identifying the receptor(s) for these viruses has been sparked by the need to define and possibly to manipulate target organs for adenovirus-mediated gene therapy. The adenoviruses, unlike CVB, do not have a broad host range and therefore encounter other restrictions in their life cycle following receptor attachment ((Shenk, T., In: *VIROLOGY,* Fields B.N. *et al.*, eds., Lippincott-Raven, Philadelphia, 1996, pp. 2111-2148). However, as, described herein, the expression of mRNA encoding HCAR, this common

receptor, in several tissues indicates that adenovirus-mediated gene deliver to a variety of tissues and organs will be possible.

Genetically-engineered chimeric receptors for Ad2, Ad5 and CVB which permit infection of a cell not otherwise infected by the above viruses would be useful in the gene therapy setting, for example, in development stage studies in mice. With the growing interest in gene therapy, there is a constant search for more efficient and safer means for introducing exogenous genes into human cells. One relatively efficient means for achieving transfer of genes is by adenovirus-mediated gene transfer (Shenk, T., *supra*; Horowitz, M.S., In Fields *et al.*, *VIROLOGY, supra,* pp. 2149-2171; Berkner, K.L., *Biotechniques 6*:616 919 (1988)); Strauss, S.E., In: *THE ADENOVIRUSES*, Ginsberg, H.S., ed., Plenum Press, New York, 1984, chapter 11).

One of the safety problems inherent in this approach, which may preclude progress in the clinic, is the fact that even viruses that have been rendered replication-defective are sometimes capable of generating wild-type variants through recombinational events. Such an alteration could lead to the possibility of widespread viral infection in cells and tissues which were not intended to be genetically modified. This could result in generalized disease. The present invention is also directed to these needs and problems.

Citation of the above documents is not intended as an admission that any of the foregoing is pertinent prior art. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicant and does not constitute any admission as to the correctness of the dates or contents of these documents.

SUMMARY OF THE INVENTION

The present inventors have isolated human cDNA and mouse cDNA that encode, respectively, the ***h***uman ***C***VB and ***A***d2 and Ad5 ***r***eceptor (HCAR), included in and the ***m***ouse ***C***VB ***A***d2 and Ad5 ***r***eceptor (MCAR). The cDNA clone which includes the HCAR coding sequence is SEQ ID NO:1 The cDNA clone which includes the MCAR encoding sequence is SEQ ID NO:3. The amino

acid sequence of HCAR is SEQ ID NO:2 and that of MCAR is SEQ ID NO:4. Both proteins encoded by these new DNA molecules have not previously been isolated in totally pure form. A human receptor for these viruses has not been characterized beyond recognition of a relationship between receptor type (biological activity with appropriate viruses)) and molecular weight or isoelectric pH. It is only with the making of the present invention that it is possibly to characterize these receptor proteins as membrane-spanning glycoproteins that contain two extracellular immunoglobulin- (Ig-) like domains and, therefore, are new members of the Ig superfamily.

The two protein molecules described herein are expected to have other biological functions than serving as virus receptor sites, although these have not yet been identified. A different human receptor for CVB has been shown to be decay accelerating factor (DAF), a complement receptor protein (Mohanty *et al.* ,*supra*: Bergelson *et al., supra.*). Based on sequence comparisons, the present inventors have found that the HCAR genetic locus localizes to human chromosome 21 and not to chromosome 19 as had been proposed by others (Couillin, P. *et al., Path. Biol. 24*:195-203 (1976).

Thus, The present invention provides an isolated DNA molecule which encodes a coxsackievirus B and Ad2 and Ad5 receptor protein or glycoprotein molecule, termed HCAR, or a protein or glycoprotein termed MCAR, or encodes a functional derivative thereof, which protein, glycoprotein or functional derivative binds group C adenoviruses or coxsackievirus B. Also intended is an allelic variant of such a DNA molecule as understood in the art.

SEQ ID NO:1 and SEQ ID NO:3 were deposited with Genbank under accession number U90716 and were put on line on 22 April 1997. SEQ ID NO:2 and SEQ ID NO:4 were deposited with Genbank under accession number U90715 and were put on line on 22 April 1997.

In one embodiment, the DNA molecule has the sequence SEQ ID NO:1, more preferably the coding sequence thereof. In another embodiment, the DNA

molecule has the sequence SEQ ID NO:3, more preferably, the coding sequence thereof.

Also included is a DNA molecule as above wherein the protein has the amino acid sequence SEQ ID NO:2 or SEQ ID NO:4.

In another embodiment, the DNA molecule is one which hybridizes under stringent conditions with the coding sequence of SEQ ID NO:1, SEQ ID NO:3 or with any stretch of twenty contiguous nucleotides of the coding sequence of SEQ ID NO:1 or SEQ ID NO:3

If the DNA molecule encodes a functional derivative, the derivative is preferably a peptide of an extracellular immunoglobulin domain of HCAR. Most preferably the peptide is selected from the group consisting of residues 35-130 of SEQ ID NO:2, and residues 155-220 of SEQ ID NO:2. Other preferred DNA molecules encode a Ad2, Ad5 or CVB binding peptides comprising one or more repeats of the sequence LSPEDQGP, PEDQG, LDIEW, QVIIL, QMIIL, ILYSGD, DKII, NDLKS, NDVKS or VKKAPG.

The above DNA molecule may be a cDNA molecule or a genomic DNA molecule.

Also provided is a DNA molecule as above which is an expression vector, such as a plasmid.

The present invention is directed to a host cell, prokaryotic or eukaryotic, most preferably mammalian, transformed or transfected with a DNA molecule as above.

In another embodiment, this invention provides a process for preparing HCAR or MCAR protein, glycoprotein or functional derivative molecule which, if naturally occurring, is substantially free of other proteins or glycoproteins with which it is natively associated, comprising culturing a host cell transformed or transfected as above under culturing conditions to express the DNA molecule and recovering the protein, glycoprotein or functional derivative molecule produced by the host cell from the culture.

The present invention is directed to HCAR or MCAR protein, glycoprotein or derivative made according to the above method.

This invention includes HCAR or MCAR protein, glycoprotein or functional derivative molecule which, if naturally occurring, is substantially free of other proteins or glycoproteins with which it is natively associated, which protein, glycoprotein or functional derivative is encoded by a DNA molecule as described above. In one embodiment, the functional derivative is a peptide of an extracellular immunoglobulin domain of HCAR, preferably a peptide selected from the group consisting of (a) residues 35-130 of SEQ ID NO:2 and (b) residues 155-220 of SEQ ID NO:2. Preferred peptides comprise one or more repeats or combinations of the sequence LSPEDQGP, PEDQG, LDIEW, QVIIL, QMIIL, ILYSGD, DKII, NDLKS, NDVKS, VKKAPG.

The present invention is also directed to a method for detecting the presence of nucleic acid encoding a normal or mutant HCAR or MCAR protein in a nucleic acid-containing sample comprising (a) contacting the sample with an oligonucleotide probe encoding at least a portion of the normal or mutant HCAR or MCAR under hybridizing conditions; and (b) measuring the hybridization of the probe to the nucleic acid of the cell, thereby detecting the presence of the nucleic acid. This method may additional comprise, before step (a), the step of selectively amplifying the DNA encoding the HCAR or MCAR protein.

Also included is a kit useful for foregoing detection method. The kit is adapted to receive therein one or more containers, and comprises a first container containing the oligonucleotide probe and a second container or plurality of containers containing a reagent or reagents capable of detecting the binding of the oligonucleotide to the sample nucleic acid.

In another embodiment is provided a method for detecting the presence or measuring the quantity of HCAR or MCAR protein, glycoprotein or functional derivative in a biological sample, comprising contacting the biological sample that is suspected of containing the protein, glycoprotein or derivative with a binding partner capable of binding to the HCAR or MCAR protein; and detecting the

binding of the binding partner to a substance in the sample or measuring the quantity of the binding partner bound, thereby determining the presence or measuring the quantity of the HCAR or MCAR protein, glycoprotein or derivative. The binding partner may be an antibody or an antigen-binding fragment thereof. In a preferred embodiment, the binding partner is a viral protein or peptide which binds to the HCAR or MCAR protein or is an HCAR or MCAR-binding functional derivative of the viral protein or peptide.

Also included is a method for identifying in a sample an analyte capable of binding to a HCAR or MCAR protein, glycoprotein or functional derivative as above, which method comprises: (a) incubating the sample which is suspected of containing the analyte in the presence of an HCAR or MCAR protein or functional derivative, or a ligand-binding portion thereof, such that the HCAR or MCAR protein, glycoprotein, derivative or portion binds to the analyte; and (b) detecting the analyte which is bound to the protein, glycoprotein, derivative or potion. In a preferred embodiment of the above method, the HCAR or MCAR protein, glycoprotein, derivative or portion is immobilized to a solid support. The analyte may be an HCAR or MCAR-binding virus, viral protein or peptide, or an HCAR or MCAR-specific antibody.

The present invention also provides a composition comprising a solid support to which is immobilized an isolated HCAR or MCAR protein, glycoprotein or functional derivative as described.

In yet another embodiment, the invention is directed to a method for isolating from a complex mixture a composition capable of binding to HCAR or MCAR protein, glycoprotein or functional derivative as described, comprising: (a) immobilizing the HCAR or MCAR protein, glycoprotein or derivative, or a ligand-binding portion thereof, to a solid support; (b) contacting the complex mixture with the solid support of step (a) so that any of the composition binds to the immobilized HCAR or MCAR protein, glycoprotein or derivative; (c) washing away any unbound material from the mixture; and (d) eluting the bound composition, thereby isolating the composition.

Also provided is a pharmaceutical composition useful for preventing or treating an infection by a virus which utilizes HCAR or MCAR as its cellular receptor, comprising a HCAR or MCAR protein, glycoprotein or functional derivative as described above; and a pharmaceutically acceptable carrier or excipient.

This invention is directed to a method for preventing or treating in a subject an infection with a virus which utilizes HCAR or MCAR as its cellular receptor, comprising administering to the subject an effective amount of the above pharmaceutical composition. In this method, the virus infection is typically with adenovirus serotype 2 or serotype 5, or a group B coxsackievirus.

Along similar lines is provided a method for inhibiting the infectivity of adenovirus serotype 2 or serotype 5, or of a group B coxsackievirus, comprising contacting the virus, *in vitro* or *in vivo* with an effective amount of the HCAR or MCAR protein molecule or derivative as above, and allowing the molecule to prevent the virus from attaching to a cell, thereby inhibiting the infectivity

Another embodiment includes a method for rendering a cell which is normally not susceptible to infection with an adenovirus susceptible to infection by, and virus-mediated gene transfer by, an adenovirus vector, comprising the steps of (a) transforming a cell of the non-susceptible species or cell type with an expression vector as described above; (b) expressing the HCAR or MCAR protein, glycoprotein or derivative on the surface of the cell, thereby rendering the cell susceptible to infection by the adenoviral vector. Also provides is a method for transferring a gene with an adenovirus vector to a cell of an animal species or a cell type normally resistant to adenovirus-mediated gene transfer, comprising (a) culturing a cell intended to receive the transferred gene; (b) rendering the cell susceptible by the above method ; and (c) infecting the cell with the adenovirus vector carrying the gene to be transferred, thereby transferring the gene.

This invention is also directed to a transgenic non-human mammal essentially all of whose germ cells and somatic cells contain a DNA sequence encoding HCAR or MCAR as described above. Preferably, the DNA molecule

has been introduced into the mammal or an ancestor of the mammal at an embryonic stage.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an electropherogram showing expression of unique RNA's found in receptor-expressing TCMK-1 cells. Total RNA from TCMK-1 or mouse L cells was probed with a ^{32}P labeled fragment of RTMCR-4 to reveal differentially expressed 6 kb (large arrow) and 1.4 kb (small arrow) RNA's.

Figures 2A and 2B show an amino acid alignment (Fig. 2A) of HCAR and MCAR and a domain model (Fig. 2B) for a CAR protein.. The deduced amino acid sequence (SEQ ID NO:2) of cloned HCAR cDNA and the deduced amino acid sequence (SEQ ID NO:4) of cloned MCAR cDNAs were aligned using the GeneWorks software program. Vertical lines (|) represent amino acid identities, dashes (-) are gaps in the alignment. The positions of the potential signal peptide (thinner underscore at the N-terminus), transmembrane region (thicker underscore), and N-linked glycosylation sites (*) are shown. In the model in Figure 2B, IG1 and IG2 represent immunoglobulin domains. SS represents the signal sequence and TM represents the transmembrane-spanning region.

Figure 3 (panels A-D), is a set of four photomicrographs showing that pRTHR and pRTMR confer susceptibility to Ad2 entry. Only TCMK-1 positive controls (panel A) or NIH3T3 cells transfected with either pRTHR (panel B) or pRTMR (panel C) stained for β-galactosidase expression following incubation with a CMV-β gal recombinant Ad5 vector. pBK-CMV controls (panel D) showed no reactivity following incubation under identical conditions.

Figures 4A and 4B show western blots of HCAR and MCAR as detected by CVB receptor-specific antibodies. 46 kDa proteins are visible in lysates from HCAR and MCAR transfected cells that are absent in pBK-CMV transfected cells. Similar sized proteins (about 46kDa) are also detectable in two positive control cell lines: HeLa and TCMK-1. Immunodetection was performed using a

mAb specific for HCAR (RmcB) (Fig. 4A, lanes 1-3) or the anti-p46 antiserum (Fig. 4B, lanes 4-6).

Figure 5 shows detection of hybridizing 6 kb and 1.4 kb mRNAs in poly A northern blots of TCMK-1 cells but not in mouse L cells. In contrast, six species of mRNA ranging in size from 6kb to 1.2kb were detected in HeLa cells that that were not found in receptor-negative rhabdomyosarcoma (Rd) cells. The sizes of the RNAs that correlate with the size of the isolated HCAR (large arrow) and MCAR (small arrow) cDNAs are indicated.

Figure 6 shows that HCAR mRNA is expressed in a variety of tissues. Human multiple tissue Northern blots were purchased from Clontech and hybridized with a ^{32}P-labeled fragment corresponding to the open reading frame of HCAR. Hybridizing sequences are similar in size to those detected in HeLa cells (Figure 4A-4B).

Figure 7 shows MCAR mRNA is expressed in multiple mouse tissues. Mouse multiple tissue Northern blots (Clontech) were hybridized with a ^{32}P-labeled fragment corresponding to the open reading frame of MCAR. Hybridizing sequences are similar in size to those detected in TCMK-1 cells (Figure 5).

Figures 8-11 are a series of views of a 3 dimensional (3D) model of the HCAR/MCAR receptor. Fig. 8 shows a 3D model of the complete HCAR/MCAR receptor. IG1 and IG2 domains were built by homology with an immunoglobulin and the CD4 protein. The C-terminal cytoplasmic Ser-Pro-Thr-rich domain was assigned an arbitrary conformation. Tyr 269 is a potential kinase phosphorylation site. Fig. 9 shows a model of a fiber knob interacting with three IG1 receptor domains. Fig. 10 shows a top view of the HCAR/MCAR receptor docked into the canyon of CVB3. Fig. 11 shows a side view of the HCAR/MCAR receptor docked into the canyon of CVB3.

Figure 12 shows the amino acid sequences or HCAR (SEQ ID NO:2) and MCAR (SEQ ID NO:4) with demarcation of the residues involved in binding to Ad2, Ad5 and/or CVB.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is directed to a DNA molecule discovered by the inventors which encodes a human virus receptor protein termed HCAR and a cDNA molecule which encodes the murine homologue termed MCAR. Collectively, these proteins are termed CAR proteins (Coxsackie and Adenovirus Receptor) . The present inventors have conceived of a method of using a CAR protein, or a functional derivative thereof, preferably a soluble form of the protein, to bind Ad2, Ad5 or CVB in a manner that prevents entry of these viruses into susceptible cells.

The methods of the present invention which identify normal or mutant CAR genes or measure the presence or amount of CAR protein associated with a cell or tissue can serve as methods for identifying susceptibility to infection by these viruses.

In one embodiment, the invention is directed to a naturally occurring HCAR or MCAR protein substantially free from impurities of human or murine origin (respectively) with which it is natively associated. In another embodiment, the invention is directed to a recombinant HCAR or MCAR protein. "Substantially free of other proteins" indicates that the protein has been purified away from at least about 90%(on a weight basis), and preferably, from at least about 99%, if desired, of other proteins and glycoproteins with which it is natively associated, and is therefore substantially free of them. By "substantially pure" is meant any protein or peptide of the present invention (or any DNA encoding any such protein or peptide), which is essentially free of other proteins (or polynucleotides) or of other contaminants with which it might normally be found in nature, and as such exists in a form not found in nature.

Such purity can be achieved by subjecting lysates or extracts of cells, tissue or fluids containing the HCAR or MCAR protein to protein purification techniques. An example of such a technique is immunoaffinity chromatography using immunoabsorbent columns bearing (1) antibodies, preferably monoclonal antibodies (mAbs) specific for the protein or (2) virus-derived ligand proteins which are the viral structures binding to the receptor protein material. Alternatively, the purification can be achieved by a combination of standard methods, such as ammonium sulfate precipitation, molecular sieve chromatography, and ion exchange chromatography.

It will be understood that the HCAR or MCAR protein of the present invention can be purified biochemically or physicochemically from a variety of cell or tissue sources. For preparation of naturally occurring HCAR protein, human pancreas or prostate tissue and cells are preferred. Alternatively, polypeptides of desired sequence may be synthesized using well-known methods employing solid phase supports.

Because the HCAR or MCAR gene (genomic DNA or cDNA) can be isolated or all or part of the coding sequence can be synthesized, the HCAR or MCAR polypeptide, or a functional derivative thereof (described in more detail below), can be prepared substantially free of other proteins or glycoproteins of mammalian origin in a prokaryotic organism or in a non-mammalian eukaryotic organism, if desired. As intended by the present invention, an HCAR or MCAR protein molecule produced by recombinant means in mammalian cells, such as transfected COS, NIH-3T3, or CHO cells, for example, is either a naturally occurring protein sequence or a functional derivative thereof. Where a naturally occurring protein or glycoprotein is produced by recombinant means, it is provided substantially free of the other proteins and glycoproteins with which it is natively associated.

A preferred use of this invention is the production by chemical synthesis or recombinant DNA technology of a fragment of the HCAR or MCAR molecule,

preferably as small as possible, while still retaining sufficient specificity and affinity of binding to CVB, Ad2 or Ad5 to act as an inhibitor of infection. Preferred fragments of HCAR or MCAR include extracellular domains IGI and IG2 (see Figures 2A and 2B) or shorter fragments of these domains which may be derived from IGI, IG2 or both. Examples of preferred peptides are shown in Table 1, below, and are indicated in the sequences shown in Figure 12. These oligopeptides of 4-8 amino acids may be used singly or in combination as inhibitors of viral infection. Furthermore, longer peptides comprising these sequences are included in this invention up to and including the full length HCAR or MCAR protein molecule. Also intended are synthetic (or recombinant) peptides which comprise one or more of these sequences. Such virus binding peptides may include between one and about 20 repeats of a single peptide sequence or various of these oligopeptides. The ordering of the peptides may be the same or different from the order in the HCAR or MCAR protein. In such synthetic peptides, spacer groups of between one and ten amino acids may be placed between the virus-binding oligopeptides described herein to permit proper folding.

TABLE 1

Preferred Virus-Binding Peptides of HCAR and MCAR Proteins

HCAR peptide	Residues of SEQ ID NO:2	**MCAR peptide**	Residues of SEQ ID NO:4	**Viruses Bound***
LSPEDQGP	45-52	LSPEDQGP	45-52	CVB
PEDQG	47-51	PEDQG	47-51	Ad2/5, CVB
LDIEW	53-57	LDIEW	53-57	Ad2/5
QVIIL	69-73	QMIIL	69-73	Ad2/5, CVB
ILYSGD	72-77	ILYSGD	72-77	CVB
DKII	77-80	DKII	77-80	Ad2/5
NDLKS	97-101	NDVKS	97-101	CVB
VKKAPG	123-128	VKKAPG	123-128	Ad2/5, CVB

* Because of similarity of binding, sites, there is either identity or overlap between peptides which bind to the two classes of virus.

Due to its activity as a virus receptor, an extracellular fragment of the HCAR or MCAR protein is expected to bind to the appropriate virus or to a viral protein or peptide which is the ligand for the cellular receptor. By production of smaller fragments of the HCAR or MCAR protein, or synthetic peptides as above, one skilled in the art, using known binding assays or binding inhibition assays, will readily be able, without undue experimentation, to identify a peptide capable of binding CVB, Ad2 or Ad5 virus with sufficiently high affinity to inhibit infectivity. Shorter peptides are expected to have two advantages over the larger proteins: (1) greater stability and diffusibility, and (2) less immunogenicity.

The identification of the HCAR or MCAR as a potential receptor or site of entry of a Ad2, Ad5 or CVB virus into target cells establishes a critical mechanism to explain how the adenovirus or CVB enters the cell. Based on the inventors' recognition of these molecules as cellular receptors, this invention provides specific HCAR or MCAR receptor "mimics" or "decoys" that can prevent viruses that use these receptors (Ad2, Ad5 or CVB) from binding to and being taken up into target cells. This permits a method for limiting initial infection and for controlling spread of an established infection by Ad2, Ad5 or CVB which would then prevent or treat virus-induced disease.

The virus infections for which the present invention is useful include Ad2, Ad5, CVB and any other virus now known or yet to be discovered which bind to HCAR or MCAR as their cellular receptor or which enter the cell via an HCAR- or MCAR-dependent mechanism.

By constructing DNA molecules encoding modified HCAR or MCAR molecules having, for example single amino acid substitutions (as well as specific substitutions of two, three or more amino acids), the invention provides an approach to systematic identification of critical sites in HCAR or MCAR which must be present for susceptibility of a cell to infection by Ad2, Ad5 or CVB. This knowledge allows development of rationally designed small molecule therapeutic agents for disrupting viral entry into target cells.

The viral receptor of the present invention can be synthesized substantially free of other proteins or glycoproteins of mammalian origin in a prokaryotic or non-mammalian eukaryotic cell. Preferably, however, the HCAR or MCAR protein molecule is produced by recombinant means and expressed in mammalian cells, most preferably in human cells. The receptor molecule of the present invention endows human cells, murine cells, or cells of other normally resistant species which are not normally capable of being infected by Ad2, Ad5 or CVB and which express the receptor with the ability to be infected by these viruses. This is particularly useful for targeted gene therapy using adenovirus vectors.

By appropriate substitutions of one or more of the amino acid residues of HCAR or MCAR, one skilled in the art, using known binding and inhibition assays, will be able, without undue experimentation, to identify the single or multiple amino acid substitutions which are responsible for binding of adenovirus vectors with sufficiently high affinity to permit infection and genetic transformation of a cell expressing HCAR or MCAR. Substitution of between about 1 and 5 residues is preferred. Substitution of as few as one amino acid may alter the virus specificity of the altered receptor protein. Another means for modifying the virus binding specificity of HCAR or MCAR is by deletion of one or more of the extracellular amino acid residues in HCAR or MCAR. Preferred deletions of between about 1 and 5 residues from HCAR or MCAR at positions 35 to 130 of the protein are preferred.

A major advantage of transfecting human cells with HCAR or murine cells with MCAR if these cells are to be used in cellular therapy or cell transfer to achieve gene therapy is the decrease in immunogenicity resulting from use of the receptor molecule native to the species.. Thus, for example, human cells normally resistant, but which are to be infected with a Ad2 or Ad5 are made to express HCAR or a functional derivative having as few amino acid residues of HCAR as necessary to confer infectibility by the virus. If cells bearing this receptor are to be introduced on multiple occasions into the same human subject, the fact that the receptor is largely of human origin decreases the chances of an undesirable

immune response which would otherwise be directed to sequences of foreign origin.

For use in *ex vivo* gene therapy, this lack of immunogenicity is important for survival and therapeutic efficacy of the infused adenovirus-infected cells. In gene therapy using bone marrow stem cells or hepatocytes, for example, it is common to manipulate the cells *in vitro* with cytokines and then to infect them with the vector bearing the gene of interest. Such cells are very short lived an have yielded very short lived therapeutic changes (see, for example, Wilson, J.M. *et al., Proc. Natl. Acad. Sci. USA 87*:8437-8441 (1990)). The addition of immunogenic epitopes on such cells would further shorten their half-life *in vivo*.

The HCAR or MCAR protein can be expressed on the cell surface as a transmembrane protein in a number of cell types, particularly cells of pancreas and liver lineages, consistent with *in vitro* tropism of Ad2, Ad5 or CVB. Thus, the protein will permit cells of these lineages in the human, which are normally resistant to Ad2, Ad5 or CVB virus infection, to be infected with an adenovirus vector.

Genetic constructs encoding HCAR or MCAR functional derivatives thereof such as those described above, can be used in gene therapy. As an example, an abnormal HCAR or MCAR molecule which results in enhanced susceptibility to disease, may be replaced by infusion of cells of the desired lineage (such as hemopoietic cells, for example) transfected with a modified HCAR or MCAR protein, under conditions where the infused cells will preferentially replace the endogenous cell population.

Adenoviruses have been recognized as useful vectors for transferring genes efficiently into human cells, for example to correct enzyme deficiencies. These viruses have varying host ranges and recognize some but not all human cells. For safety reasons, it is important that an adenoviral vector used for gene therapy be capable of infecting only desired cells and not cause generalized infection of cells throughout the body of the individual being treated. In the past,

this has generally been accomplished by using defective virus preparations, or mutants lacking the E1 and E3 regions of the viral genome. Even in murine model work, it is important that the mouse be protected from generalized infection. The present invention provides an additional and improved measure of safety compared to the prior art approaches in that it permits use of murine cells or transgenic mice with a selected susceptibility to the virus. Only those cells to be infected with the vector, or cells of a particular lineage in which the receptor is expressed, for example by differential choice of promoter or enhancer sequences, are given the capacity to be infected and genetically altered by virtue of their expression of the CAR of this invention.

While gene transfer using adenovirus vectors is generally more efficient than transfection with naked DNA, some cells are not easily infectible by these viruses , making it difficult to use adenoviruses as vectors for introducing new or altered genes into such cells. According to the present invention, a human cell which is not infectable by a Ad2, Ad5 or CVB virus or is infectable only at very low efficiency due to lack of sufficient receptor protein on its surface is transfected with the HCAR gene or a functional derivative, and the HCAR protein (or functional derivative) is expressed, resulting in Ad2, Ad5 or CVB virus receptor appearing on the cell surface. This approach can be used both in cells expressing no CAR or less CAR than is desirable for optimal gene transfer by the viral vector. Such a transfected cell can then be infected with an Ad2, Ad5 or CVB vector carrying a gene of interest, in order to transfer the gene of interest permanently (or transiently, if desired) into the cell.

For a general discussion of adenovirus-mediated gene transfer, see, for example, Horowitz, M.S., *supra*; Berkner, K.L., *supra*; Strauss, S.E., *supra*). Advantages of adenovirus vectors for human gene therapy include the fact that no human malignancies are known to be associated with such viruses, the adenovirus genome is double stranded DNA which can be manipulated to accept foreign genes of up to 7.5 kb in size, and live adenovirus is a safe human vaccine entity.

The present invention is intended to encompass Ad2, Ad5 or CVB viruses, or any other mammalian virus with similar receptor specificity, which attaches to the HCAR or MCAR molecule of the present invention as its cellular receptor or enters the cell via an HCAR-or MCAR-dependent mechanism.

More broadly, the invention is directed to the general concept of generating a viral receptor which will allow selected cells or cells of a selected animal species to be infected with a Ad2, Ad5 or CVB virus which normally does not infect cells of that type or species. Examples of cells which are relatively resistant to CVB infection include WI-38 cells and other fibroblast-type cell lines such as various strains of NIH-3T3 mouse fibroblasts, mouse L cells, various strains of Chinese hamster ovary (CHO))saroma origin and RD cells originating from human rhabdomyosarcoma. .

One of ordinary skill in the art will know how to obtain DNA encoding a protein homologous to HCAR or MCAR from any species or cell type without undue experimentation. First, one screens (using methods routine in the art) a cDNA library of the species or cell type of interest using a probe based on the sequence of HCAR or MCAR. Next, one will clone and sequence the hybridizing DNA to obtain the sequence of the "new" viral receptor. By visual inspection or with the aid of a computer program (as described herein) it is possible to identify the regions in which the sequence of the new viral receptor protein differs from HCAR or MCAR. In particular, one will concentrate on the extracellular domain region. Based on the sequence differences observed, it is possible, using the teachings provided herein, to create a sequence having one or more amino acid substitutions such that a chimeric receptor between the new receptor and a known receptor is created. The chimeric receptor can then be expressed in a cell of choice and its function can easily be tested using conventional virus binding assays or virus infectivity assays.

Furthermore, according to the present invention, it is possible to modify the receptor attachment site of a virus so that it will not bind to its natural

receptor. For example, changes in the DNA sequence of Ad2 or Ad5 will render these viruses non-infective for HCAR-bearing cells. Corresponding changes may be introduced into HCAR so that this mutant adenovirus will bind to it. In this way, a safer adenovirus preparation can be generated which binds only to select cells bearing the appropriate mutant/variant receptor, but not to the normal targets of adenovirus.

It is also within the scope of the present invention to express more than a single type of intact, functional derivative, or chimeric HCAR or MCAR molecule on the surface of the same cell. Thus, by virtue of a first "normal" or mutant HCAR, a human cell can be infected with "normal" or a corresponding mutant adenovirus *in vitro* in a transient fashion, and can be manipulated by the judicious use of cytokine growth or differentiation factors. Such cells can be introduced into a recipient. At the desired time, a second virus which binds to a second genetically engineered receptor can be introduced into the individual to infect stably alter only those introduced cells bearing the second viral receptor.

The preferred animal subject of the present invention is a mammal. The invention is particularly useful in the treatment of human subjects, although it is intended for veterinary uses as well.

As discussed above, the present invention includes soluble forms of HCAR or MCAR (or chimeric receptors), as well as functional derivatives thereof having similar bioactivity for all the uses described herein. Also intended are all active forms of HCAR or MCAR derived from the HCAR or MCAR transcript, and all muteins with HCAR or MCAR activity. Methods for production of soluble forms of receptors which are normally transmembrane proteins are well known in the art (see, for example, Smith, D.H. *et al.*, *Science 238*:1704-1707 (1987); Fisher, R.A. *et al.*, *Nature 331*:76-78 (1988); Hussey, R.E. *et al.*, *Nature 331*:78-81 (1988); Deen, K.C. *et al.*, *Nature 331*:82-84 (1988); Traunecker, A. *et al.*, *Nature 331*:84-86 (1988); Gershoni, J.M. *et al.*, *Proc. Natl. Acad. Sci. USA 85*:4087-4089 (1988), which references are hereby incorporated by reference).

Such methods are generally based on truncation of the DNA encoding the receptor protein to exclude the transmembrane portion, leaving intact the extracellular domain (or domains) capable of interacting with specific ligands, such as an intact Ad2, Ad5 or CVB virus or a adenoviral protein or glycoprotein.

For the purposes of the present invention, it is important that the soluble HCAR or MCAR, or a functional derivative of HCAR or MCAR, comprise the elements of the binding site of the HCAR or MCAR that permits binding to a Ad2, Ad5 or CVB virus. Of the many amino acid residues of HCAR or MCAR, only a few are critically involved in virus recognition and binding. The HCAR or MCAR proteins or peptides of the present invention may be further modified for purposes of drug design, such as, for example, to reduce immunogenicity, to promote solubility or enhance delivery, or to prevent clearance or degradation.

"Functional derivative" of the HCAR or MCAR protein is defined as a "fragment," "variant," "analogue," or "chemical derivative" of the HCAR or MCAR protein. A functional derivative retains at least a portion of the function of the HCAR or MCAR protein which permits its utility in accordance with the present invention, preferably the capacity to bind to Ad2, Ad5, CVB or to a viral protein or glycoprotein which is responsible for virus binding to the receptor.

A "fragment" of the HCAR or MCAR protein is any subset of the molecule, that is, a shorter peptide.

A "variant" of the HCAR or MCAR refers to a molecule substantially similar to either the entire protein or fragment thereof. Variant peptides may be conveniently prepared by direct chemical synthesis of the variant peptide, using methods well- known in the art.

Alternatively, amino acid sequence variants of the peptide can be prepared by mutations in the DNA which encodes the synthesized peptide. Such variants include, for example, deletions from, or insertions or substitutions of, residues within the amino acid sequence. Any combination of deletion, insertion, and substitution may also be made to arrive at the final construct, provided that the

final construct possesses the desired activity. Obviously, the mutations that will be made in the DNA encoding the variant peptide must not alter the reading frame and preferably will not create complementary regions that could produce secondary mRNA structure (see European Patent Publication No. EP 75,444).

At the genetic level, these variants ordinarily are prepared by site-directed mutagenesis (as exemplified by Adelman *et al.*, *DNA* 2:183 (1983)) of nucleotides in the DNA encoding the HCAR or MCAR molecule or peptide , thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Preferred variants exhibit the same qualitative biological activity as the nonvariant peptide. In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector that includes within its sequence a DNA sequence that encodes the relevant peptide. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically, for example, by the method of Crea *et al.*, *Proc. Natl. Acad. Sci. (USA)* 75:5765 (1978). This primer is then annealed with the single-stranded protein-sequence-containing vector, and subjected to DNA-polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, to complete the synthesis of the mutant strand. Thus, a mutated sequence in the second strand bears the desired mutation. This heteroduplex vector is used to transform appropriate cells, and clones are selected that include recombinant vectors bearing the mutant sequence. The mutant sequence encoding the mutant protein region may be removed and placed in an appropriate vector for protein expression in appropriate host cells.

One type of variant involves a terminal insertion such that a signal sequence, whether heterologous or homologous to the host cell is fused to the N-terminus of the peptide molecule to facilitate the secretion of mature peptide molecule from recombinant hosts. The natural signal sequences of HCAR or MCAR, as shown in Figure 2A may be used for this purpose.

One group of variants, substitution variants, comprises a protein or peptide in which at least one amino acid residue, and preferably, only one, has been removed and a different residue inserted in its place. For a detailed description of protein chemistry and structure, see Schulz, G.E. *et al.*, *Principles of Protein Structure*, Springer-Verlag, New York, 1978, and Creighton, T.E., *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, 1983, which are hereby incorporated by reference. The types of substitutions selected may be based, for example, on the frequencies of amino acid changes between a homologous protein of different species, such as those presented in Table 1-2 of Schulz *et al.* (*supra*) and Figure 3-9 of Creighton (*supra*). Conservative substitutions are defined herein as exchanges within one of the following five groups:

1. Small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr (Pro, Gly);
2. Polar, negatively charged residues and their amides: Asp, Asn, Glu, Gln;
3. Polar, positively charged residues: His, Arg, Lys;
4. Large aliphatic, nonpolar residues: Met, Leu, Ile, Val (Cys); and
5. Large aromatic residues: Phe, Tyr, Trp.

Pro, Gly and Cys have special roles in protein architecture. Gly is the only residue lacking any side chain and imparts flexibility to the chain. Pro, because of its unusual geometry, tightly constrains the chain. Cys participates in disulfide bond formation which is important for protein folding. Tyr, because of its hydrogen bonding potential, has some kinship with Ser, Thr, *etc.*

More substantial changes in functional or immunological properties are made by selecting substitutions that are less conservative, such as between, rather than within, the above five groups, which will differ more significantly in their effect on maintaining (a) the structure of the peptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or

hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Examples of such substitutions are (a) substitution of Gly and/or Pro by another amino acid or deletion or insertion of Gly or Pro; (b) substitution of a hydrophilic residue, *e.g.*, Ser or Thr, for (or by) a hydrophobic residue, *e.g.*, Leu, Ile, Phe, Val or Ala; (c) substitution of a Cys residue for (or by) any other residue; (d) substitution of a residue having an electropositive side chain, *e.g.*, Lys, Arg or His, for (or by) a residue having an electronegative charge, *e.g.*, Glu or Asp; or (e) substitution of a residue having a bulky side chain, *e.g.*, Phe, for (or by) a residue not having such a side chain, *e.g.*, Gly.

Most deletions and insertions, and substitutions according to the present invention are those which do not produce radical changes in the characteristics of the protein or peptide molecule. However, when it is difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect is evaluated by routine screening assays, either immunoassays or bioassays. For example, a variant typically is made by site-specific mutagenesis of the peptide-encoding nucleic acid, expression of the variant nucleic acid in recombinant cell culture, and, optionally, purification of the protein or peptide from the cell culture supernatant or cell extracts, for example, by immunoaffinity chromatography using an immobilized antibody specific for the or the viral ligand for the receptor on a column.

The activity of a cell lysate containing HCAR or MCAR or a functional derivative thereof, or a purified preparation of HCAR or MCAR, can be screened in a suitable screening assay for the desired characteristic. For example, a change in the immunological character of the protein molecule expressed as altered binding to a given antibody, is measured by a competitive type immunoassay (see below). Biological activity is screened in an appropriate bioassay, such as virus binding or infectivity, as described herein.

Modifications of peptide properties such as redox or thermal stability, hydrophobicity, susceptibility to proteolytic degradation or the tendency to

aggregate with carriers or into multimers are assayed by methods well known to the ordinarily skilled artisan.

An "analogue" of the HCAR or MCAR protein refers to a non-natural molecule substantially similar to either the entire molecule or a fragment thereof.

A "chemical derivative" of the HCAR or MCAR protein contains additional chemical moieties not normally a part of the protein. Covalent modifications of the peptide are included within the scope of this invention. Such modifications may be introduced into the molecule by reacting targeted amino acid residues of the peptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues.

Cysteinyl residues most commonly are reacted with alpha-haloacetates (and corresponding amines), such as 2-chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, alpha-bromo- beta-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N- alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4- nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

Histidyl residues are derivatized by reaction with diethylprocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1 M sodium cacodylate at pH 6.0.

Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing alpha-amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4 pentanedione; and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3- butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pK_a of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

The specific modification of tyrosyl residues has been studied extensively, with particular interest in introducing spectral labels by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidizol and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively.

Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R'-N-C-N-R') such as 1-cyclohexyl-3-(2-morpholinyl-(4-ethyl) carbodiimide or 1- ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

Derivatization with bifunctional agents is useful for cross-linking the peptide to a water-insoluble support matrix or to other macromolecular carriers. Commonly used cross-linking agents include, *e.g.*, 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'- dithiobis(succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light.

Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates described in U.S. Patents 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α-amino groups of lysine, arginine, and histidine side chains (Creighton, *supra*), acetylation of the N-terminal amine or amidation of the C-terminal carboxyl groups.

Such derivatized moieties may improve the solubility, absorption, biological half life, and the like. The moieties may alternatively eliminate or attenuate any undesirable side effect of the protein and the like. Moieties capable of mediating such effects are disclosed, for example, in *Remington's Pharmaceutical Sciences*, 16th ed., Mack Publishing Co., Easton, PA (1980).

Standard reference works setting forth the general principles of recombinant DNA technology include Watson, J.D. *et al.*, *Molecular Biology of the Gene*, Volumes I and II, The Benjamin/Cummings Publishing Company, Inc., publisher, Menlo Park, CA (1987); Darnell, J.E. *et al.*, *Molecular Cell Biology*, Scientific American Books, Inc., publisher, New York, N.Y. (1986); Lewin, B.M., *Genes II*, John Wiley & Sons, publishers, New York, N.Y. (1985); Old, R.W., *et al.*, *Principles of Gene Manipulation: An Introduction to Genetic Engineering*, 2d edition, University of California Press, publisher, Berkeley, CA (1981); and Sambrook, J. *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989). These references are hereby incorporated by reference.

The recombinant DNA molecules of the present invention can be produced through any of a variety of means, such as, for example, DNA or RNA synthesis, or more preferably, by application of recombinant DNA techniques. By "cloning" is meant the use of *in vitro* recombination techniques to insert a particular gene or

other DNA sequence into a vector molecule. In order to successfully clone a desired gene, it is necessary to employ methods for generating DNA fragments, for joining the fragments to vector molecules, for introducing the composite DNA molecule into a host cell in which it can replicate, and for selecting the clone having the target gene from amongst the recipient host cells.

By "cDNA" is meant complementary or copy DNA produced from an RNA template by the action of RNA-dependent DNA polymerase (reverse transcriptase). Thus a "cDNA clone" means a duplex DNA sequence complementary to an RNA molecule of interest, carried in a cloning vector. A "cDNA library" is a collection of recombinant DNA molecules containing cDNA inserts which together comprise the entire expressible genome of an organism. Such a cDNA library may be prepared by methods known to those of skill, and described, for example, in Sambrook *et al.*, *supra*. Generally, RNA is first isolated from the cells of an organism from whose genome it is desired to clone a particular gene. Preferred for the purposes of the present invention are mammalian cell lines.

Oligonucleotides representing a portion of the HCAR or MCAR sequence are useful for screening for the presence of homologous genes and for the cloning of such genes. Techniques for synthesizing such oligonucleotides are disclosed by, for example, Wu, R., *et al.*, *Prog. Nucl. Acid. Res. Molec. Biol. 21*:101-141 (1978)).

The oligonucleotide, or set of oligonucleotides, containing the theoretical "most probable" sequence capable of encoding an HCAR or MCAR fragment is used to identify the sequence of a complementary oligonucleotide or set of oligonucleotides which is capable of hybridizing to the "most probable" sequence, or set of sequences. An oligonucleotide containing such a complementary sequence can be employed as a probe to identify and isolate the HCAR or MCAR gene or a yet unidentified homologous gene (Sambrook *et al.*, *supra*). A suitable oligonucleotide, or set of oligonucleotides, which is capable of encoding a

fragment of the HCAR or MCAR gene (or which is complementary to such an oligonucleotide, or set of oligonucleotides) is identified, synthesized, and hybridized by means well known in the art, against a DNA or, more preferably, a cDNA preparation derived from cells which are capable of expressing the HCAR or MCAR gene or putative homologue. Single stranded oligonucleotide molecules complementary to the "most probable" HCAR or MCAR peptide coding sequences can be synthesized using procedures which are well known to those of ordinary skill in the art (Belagaje, R., *et al.*, *J. Biol. Chem. 254*:5765-5780 (1979); Maniatis, T., *et al.*, *In*: *Molecular Mechanisms in the Control of Gene Expression*, Nierlich, D.P., *et al.*, Eds., Acad. Press, NY (1976); Wu, R., *et al.*, *Prog. Nucl. Acid Res. Molec. Biol. 21*:101-141 (1978); Khorana, R.G., *Science 203*:614-625 (1979)). DNA synthesis may be achieved through the use of automated synthesizers. Techniques of nucleic acid hybridization are disclosed by Sambrook *et al.* (*supra*), and by Haymes, B.D., *et al.* (In: *Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, DC (1985)).

In an alternative way of cloning the HCAR or MCAR gene is expression cloning, wherein a library of expression vectors is prepared by cloning DNA, preferably, cDNA (from a cell capable of expressing HCAR or MCAR) into an expression vector. The library is screened for members capable of expressing a protein which binds, for example, to an anti-HCAR or anti-MCAR antibody or a viral protein ligand, and which therefore has a nucleotide sequence capable of encoding polypeptides that have the same sequence as HCAR or MCAR proteins or peptides, or fragments thereof. In this embodiment, genomic DNA or cDNA is fragmented (by shearing, endonuclease digestion, *etc.*) to produce a pool of DNA fragments which are cloned into an expression vector in order to produce a genomic library (or cDNA library) of expression vectors whose members each contain a unique cloned DNA fragment. Procedures for preparing cDNA and for producing a genomic library are disclosed by Sambrook *et al.* (*supra*).

A "vector" is a DNA molecule, derived from a plasmid or bacteriophage or animal virus, into which selected fragments of DNA may be inserted or cloned. A

vector will contain one or more unique restriction sites, and may be capable of autonomous replication or integration into the DNA of its host such that the cloned sequence is reproducible. An "expression vector" is a vector which (due to the presence of appropriate transcriptional and/or translational control sequences) is capable of expressing a DNA (or cDNA) molecule which has been cloned into the vector and of thereby producing the encoded peptide or protein. Expression of the cloned sequences occurs when the expression vector is introduced into an appropriate host cell. If a prokaryotic expression vector is employed, then the appropriate host cell would be any prokaryotic cell capable of expressing the cloned sequences. Similarly, if a eukaryotic expression vector is employed, then the appropriate host cell would be any eukaryotic cell capable of expressing the cloned sequences.

Also included in this invention is a nucleic acid "functional derivative" which is defined as a polynucleotide or oligonucleotide which encodes a "fragment" "variant" or "analogue" of the native CAR protein. Such a functional derivative may be "substantially similar" in nucleotide sequence to the CAR-encoding sequence and thus encode a protein possessing similar activity to the native protein. A "functional derivative" of a polynucleotide can also be a chemical derivative which retains its functions. Such a chemical derivative is useful as a molecular probe to detect CAR-encoding sequences in nucleic acid hybridization assays.

A molecule is said to be "substantially similar" to another molecule if the sequence of amino acids or nucleotides in both molecules is substantially the same. Substantially similar protein or peptide molecules will possess a similar biological activity. Thus, provided that two molecules possess a similar activity, they are considered variants as that term is used herein even if one of the molecules contains additional amino acid residues not found in the other, or if the sequence of amino acid residues is not identical.

A DNA sequence encoding the CAR protein or functional derivative may be recombined with vector DNA in accordance with conventional techniques, including blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for such manipulations are disclosed by Sambrook, J. *et al.*, *supra*, and are well known in the art.

A nucleic acid molecule, such as DNA, is "capable of expressing" a polypeptide if (a) it contains a nucleotide sequence which includes transcriptional and translational regulatory information and (b) such sequences are "operably linked" to the coding sequence. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene expression. The precise nature of the regulatory regions needed for gene expression may vary from organism to organism, but shall in general include a promoter region (which in prokaryotes contains both the promoter as well as the DNA sequences which, when transcribed into RNA, will signal the initiation of protein synthesis). Such regions will normally include those 5'- non-coding sequences involved with initiation of transcription and translation, such as the TATA box, capping sequence, CAAT sequence, and the like. If desired, the non-coding region 3' to the coding sequence may be obtained by the above- described methods. This region may be retained for its transcriptional termination regulatory sequences, such as termination and polyadenylation. Thus, by retaining the 3'-region naturally contiguous to the DNA coding sequence , the transcriptional termination signals may be provided. Where the transcriptional termination signals are not satisfactorily functional in the expression host cell, then a different 3' region functional in the host cell may be substituted.

Two sequences of a nucleic acid molecule are said to be "operably linked" when they are linked to each other in a manner which either permits both sequences to be transcribed onto the same RNA transcript, or permits an RNA

transcript, begun in one sequence to be extended into the second sequence. Thus, two sequences, such as a promoter sequence and any other "second" sequence of DNA or RNA are operably linked if transcription commencing in the promoter sequence will produce an RNA transcript of the operably linked second sequence. In order to be "operably linked" it is not necessary that two sequences be immediately adjacent to one another.

The promoter sequences of the present invention may be either prokaryotic, eukaryotic or viral. Suitable promoters are repressible, or, more preferably, constitutive. Strong promoters are preferred.

The present invention encompasses the expression of the HCAR or MCAR protein (or a functional derivative thereof) or a chimeric HCAR or MCAR protein in either prokaryotic or eukaryotic cells, although preferred expression is in eukaryotic cells, most preferably in human cells.

To express the CAR protein of the present invention in a prokaryotic cell (such as, for example, *E. coli*, *B. subtilis*, *Pseudomonas*, *Streptomyces*, *etc.*), it is necessary to operably link the HCAR or MCAR encoding sequence to a functional prokaryotic promoter, examples of which are well-known in the art. Examples of constitutive promoters include the *int* promoter of bacteriophage λ, the *bla* promoter of the β-lactamase gene of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene of pBR325, *etc.* Examples of inducible prokaryotic promoters include the major right and left promoters of bacteriophage l (P_L and P_R), the *trp*, *recA*, *lacZ*, *lacI*, and *gal* promoters of *E. coli* (reviewed in Glick, B.R., *J. Ind. Microbiol. 1*:277-282 (1987); Cenatiempo, Y. *Biochimie 68*:505-516 (1986); Gottesman, S. (*Ann. Rev. Genet. 18*:415-442 (1984).

Eukaryotic hosts include yeast, insects, fungi, and mammalian cells either *in vivo*, or in tissue culture. Mammalian cells provide post-translational modifications to protein molecules including correct folding or glycosylation at correct sites. Mammalian cells which may be useful as hosts include cells of fibroblast origin such as VERO or CHO, or cells of lymphoid origin, such as the

hybridoma SP2/O-Ag14 or the murine myeloma P3-X63Ag8, and their derivatives. Preferred mammalian cells are cells which are intended to replace the function of the genetically "deficient" cells *in vivo*.

Preferred eukaryotic promoters include the promoter of the mouse metallothionein I gene (Hamer, D., *et al.*, *J. Mol. Appl. Gen. 1*:273-288 (1982)); the TK promoter of Herpes virus (McKnight, S., *Cell 31*:355-365 (1982)); the SV40 early promoter (Benoist, C., *et al.*, *Nature (London) 290*:304-310 (1981)); the yeast *gal4* gene promoter (Johnston, S.A., *et al.*, *Proc. Natl. Acad. Sci. (USA) 79*:6971-6975 (1982); Silver, P.A., *et al.*, *Proc. Natl. Acad. Sci. (USA) 81*:5951-5955 (1984)). For a mammalian cell host, many possible vector systems are well-known and commercially available for the expression of HCAR or MCAR. A wide variety of transcriptional and translational regulatory sequences may be employed, depending upon the nature of the host. The transcriptional and translational regulatory signals may be derived from viral sources, such as adenovirus, bovine papilloma virus, Simian virus, or the like, where the regulatory signals are associated with a particular gene which has a high level of expression. Alternatively, promoters from mammalian expression products, such as actin, collagen, myosin, *etc.*, may be employed. Transcriptional initiation regulatory signals may be selected which allow for repression or activation, so that expression of the genes can be modulated. Of interest are regulatory signals which are temperature-sensitive so that by varying the temperature, expression can be repressed or initiated, or are subject to chemical regulation, *e.g.*, metabolite.

For yeast cell hosts, any of a series of yeast gene expression systems can be utilized which incorporate promoter and termination elements from the actively expressed genes coding for glycolytic enzymes produced in large quantities when yeast are grown in glucose-rich medium. Known glycolytic genes can also provide very efficient transcriptional control signals. For example, the promoter and terminator signals of the phosphoglycerate kinase gene can be utilized. Production of HCAR or MCAR molecules in insects can be achieved, for example, by infecting the insect host with a baculovirus engineered to express

HCAR or MCAR by methods known to those of skill (Jasny, *Science 238*: 1653 (1987).

The HCAR or MCAR coding sequence and an operably linked promoter may be introduced into a recipient prokaryotic or eukaryotic cell either as a non-replicating DNA (or RNA) molecule, which may either be a linear molecule or, more preferably, a closed covalent circular molecule. Since such molecules are incapable of autonomous replication, the expression of the HCAR or MCAR protein may occur through the transient expression of the introduced sequence. Alternatively, permanent expression may occur through the integration of the introduced sequence into the host chromosome. In one embodiment, a vector is employed which is capable of integrating the desired gene sequences into the host cell chromosome. Cells which have stably integrated the introduced DNA into their chromosomes can be selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector. The marker may provide for prototropy to an auxotrophic host, biocide resistance, *e.g.*, antibiotics, or heavy metals, such as copper or the like. The selectable marker gene can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection. Additional elements may also be needed for optimal synthesis of single chain binding protein mRNA. These elements may include splice signals, as well as transcription promoters, enhancers, and termination signals. cDNA expression vectors incorporating such elements include those described by Okayama, H., *Mol. Cell. Biol. 3*:280 (1983).

In another embodiment, the introduced sequence will be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors may be employed for this purpose. Preferred eukaryotic plasmids include BPV, vaccinia, SV40, 2-micron circle, *etc.*, or their derivatives. Such plasmids are well known in the art (Botstein, D., *et al.*, *Miami Winter. Symp. 19*:265-274 (1982); Broach, J.R., In: *The Molecular Biology of the Yeast Saccharomyces,* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 445-470 (1981); Broach, J.R., *Cell 28*:203-204 (1982); Bollon, D.P., *et al.*, *J.*

Clin. Hematol. Oncol. 10:39-48 (1980); Maniatis, T., In: *Cell Biology: A Comprehensive Treatise, Vol. 3,* Academic Press, NY, pp. 563-608 (1980)). Preferred vectors for transient expression of the HCAR or MCAR in CHO cells is the pSG5 or pCDM8 expression vector.

Once the vector or DNA sequence containing the construct(s) has been prepared for expression, the vector or DNA construct(s) may be introduced into an appropriate host cell by any of a variety of suitable means, including such biochemical means as transformation, transfection, conjugation, protoplast fusion, calcium phosphate-precipitation, and application with polycations such as diethylaminoethyl (DEAE) dextran, and such mechanical means as electroporation, direct microinjection, and microprojectile bombardment (Johnston *et al.*, *Science 240*:1538 (1988)), *etc.* After the introduction of the vector, recipient cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene sequence(s) results in the production of HCAR or MCAR or the chimeric HCAR or MCAR protein and its expression on the cell surface.

If so desired, the expressed HCAR or MCAR protein may be isolated and purified in accordance with conventional conditions, such as extraction, precipitation, chromatography, affinity chromatography, electrophoresis, or the like. For example, the cells may be collected by centrifugation, or with suitable buffers, lysed, and the protein isolated by column chromatography, for example, on DEAE-cellulose, phosphocellulose, polyribocytidylic acid-agarose, hydroxyapatite or by electrophoresis or immunoprecipitation.

Furthermore, manipulation of the genetic constructs of the present invention allow the grafting of the Ad2, Ad5 or CVB virus binding domain of HCAR or MCAR onto the transmembrane and intracytoplasmic portions of another molecule, resulting in yet another type of chimeric molecule.

The present invention is also directed to a transgenic non-human eukaryotic animal (preferably a rodent, such as a mouse) the germ cells and

somatic cells of which contain genomic DNA according to the present invention which codes for the HCAR or MCAR protein or a functional derivative thereof capable as serving as a human Ad2, Ad5 or CVB virus receptor. The HCAR or MCAR DNA is introduced into the animal to be made transgenic, or an ancestor of the animal, at an embryonic stage, preferably the one-cell, or fertilized oocyte, stage, and generally not later than about the 8-cell stage. The term "transgene," as used herein, means a gene which is incorporated into the genome of the animal and is expressed in the animal, resulting in the presence of protein in the transgenic animal.

There are several means by which such a gene can be introduced into the genome of the animal embryo so as to be chromosomally incorporated and expressed. One method is to transfect the embryo with the gene as it occurs naturally, and select transgenic animals in which the gene has integrated into the chromosome at a locus which results in expression. Other methods for ensuring expression involve modifying the gene or its control sequences prior to introduction into the embryo. One such method is to transfect the embryo with a vector (see above) containing an already modified gene. Other methods are to use a gene the transcription of which is under the control of a inducible or constitutively acting promoter, whether synthetic or of eukaryotic or viral origin, or to use a gene activated by one or more base pair substitutions, deletions, or additions (see above).

Introduction of the desired gene sequence at the fertilized oocyte stage ensures that the transgene is present in all of the germ cells and somatic cells of the transgenic animal and has the potential to be expressed in all such cells. The presence of the transgene in the germ cells of the transgenic "founder" animal in turn means that all its progeny will carry the transgene in all of their germ cells and somatic cells. Introduction of the transgene at a later embryonic stage in a founder animal may result in limited presence of the transgene in some somatic cell lineages of the founder; however, all the progeny of this founder animal that

inherit the transgene conventionally, from the founder's germ cells, will carry the transgene in all of their germ cells and somatic cells.

Chimeric non-human mammals in which fewer than all of the somatic and germ cells contain the HCAR or MCAR DNA of the present invention, such as animals produced when fewer than all of the cells of the morula are transfected in the process of producing the transgenic mammal, are also intended to be within the scope of the present invention. The techniques described in Leder, U.S. Patent 4,736,866 (hereby incorporated by reference) for producing transgenic non-human mammals may be used for the production of the transgenic non-human mammal of the present invention. The various techniques described in Palmiter, R. *et al.*, *Ann. Rev. Genet. 20*:465-99 (1986), the entire contents of which are hereby incorporated by reference, may also be used.

The animals carrying the HCAR or MCAR gene can be used to test compounds or other treatment modalities which may prevent, suppress or cure a human Ad2, Ad5 or CVB virus infection or a disease resulting from such infection for those Ad2, Ad5 or CVB viruses which infect the cells using the HCAR or MCAR molecule as a receptor. These tests can be extremely sensitive because of the ability to adjust the virus dose given to the transgenic animals of this invention. Such animals will also serve as a model for testing of diagnostic methods for the same human Ad2, Ad5 or CVB virus diseases. Transgenic animals according to the present invention can also be used as a source of cells for cell culture.

This invention is also directed to an antibody specific for an epitope of HCAR or MCAR protein. In additional embodiments, this antibody is used to prevent or treat Ad2, Ad5 or CVB virus infection, to detect the presence of, or measure the quantity or concentration of, HCAR or MCAR protein in or on the surface of a cell, or in a cell or tissue extract, or a biological fluid.

The term "antibody" is meant to include polyclonal antibodies and monoclonal antibodies (mAbs) (Kohler and Milstein, *Nature 256*:495-497 (1975);

U.S. Patent 4,376,110); Hartlow, E. *et al.*, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988); *Monoclonal Antibodies and Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, New York, NY (1980)); Campbell, A., In: *Laboratory Techniques in Biochemistry and Molecular Biology*, Volume 13 (Burdon, R., *et al.*, eds.), Elsevier, Amsterdam (1984). A mAb may be of any immunoglobulin class including IgG, IgM, IgE, IgA and any subclass thereof. The hybridoma producing the mAbs of this invention may be cultivated *in vitro* or *in vivo*. Production of high titers of mAbs *in vivo* production makes this the presently preferred method of production, and mAbs of isotype IgM or IgG may be purified from ascites fluids, or from culture supernatants, using column chromatography methods well known to those of skill in the art. Also intended are anti-idiotypic antibodies (*Idiotypy in Biology and Medicine*, Academic Press, New York, 1984; *Immunological Reviews* Volume 79, 1984; *Immunological Reviews* Volume 90, 1986; *Curr. Top. Microbiol., Immunol.* Volume 119, 1985; Bona, C. *et al.*, *CRC Crit. Rev. Immunol.*, pp. 33-81 (1981)). This invention also includes humanized antibodies or chimeric antibodies (Morrison *et al.*, *Proc. Natl. Acad. Sci. USA 81*:6851-6855 (1984); Neuberger *et al.*, *Nature 314*:268-270 (1985); Sun *et al.*, *Proc. Natl. Acad. Sci. USA 84*:214-218 (1987); Better *et al.*, *Science 240*:1041-1043 (1988); Better, M.D. WO 91/07494).

The term "antibody" is also meant to include both intact molecules as well as fragments thereof, such as, for example, Fab and $F(ab')_2$, which are capable of binding antigen. Fab and $F(ab')_2$ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl *et al.*, *J. Nucl. Med. 24*:316-325 (1983)). It will be appreciated that Fab and $F(ab')_2$ and other fragments of the antibodies useful in the present invention may be used for the detection and quantitation of HCAR or MCAR protein according to the methods disclosed herein for intact antibody molecules. Such fragments are typically produced by

proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments).

Antibody molecules or fragments may be purified by known techniques, *e.g.*, immunoabsorption or immunoaffinity chromatography (Hartlow *et al.*, *supra*), chromatographic methods such as HPLC (high performance liquid chromatography), or a combination thereof, *etc.*

The antibodies, or fragments of antibodies, of the present invention may be used to quantitatively or qualitatively detect the presence of cells which express HCAR or MCAR protein (or a chimeric receptor having an HCAR or MCAR-derived epitope) on their surface or intracellularly. This can be accomplished by immunofluorescence techniques employing a fluorescently labeled antibody (see below) coupled with light microscopic, flow cytometric, or fluorimetric detection. The antibodies of the present invention may be employed histologically, as in immunofluorescence or immunoelectron microscopy, for *in situ* detection of HCAR or MCAR protein. *In situ* detection may be accomplished by removing a histological (cell or tissue) specimen from a subject and providing the a labeled antibody of the present invention to such a specimen. Through the use of such a procedure, it is possible to determine not only the presence of the HCAR or MCAR protein but also its distribution on the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

Additionally, the antibody of the present invention can be used to detect the presence of soluble HCAR or MCAR molecules in a biological sample. Used in this manner, the antibody can serve as a means to monitor the presence and quantity of HCAR or MCAR proteins or derivatives used therapeutically in a subject to prevent or treat human Ad2, Ad5 or CVB virus infection.

Immunoassays for HCAR or MCAR protein typically comprise incubating a biological sample, such as a biological fluid, a tissue extract, freshly harvested

cells such as lymphocytes or leucocytes, or cells which have been incubated in tissue culture, in the presence of a detectably labeled antibody capable of identifying HCAR or MCAR protein, and detecting the antibody by any of a number of techniques well-known in the art.

The biological sample may be contacted with a solid phase support or carrier (which terms are used interchangeably herein) such as nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled CAR-specific antibody. The solid phase support may then be washed with the buffer to remove unbound antibody. The amount of bound label on the solid support may then be detected by conventional means.

By "solid phase support" or "carrier" is intended any support capable of binding an antigen or an antibody. Well-known supports, or carriers, include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, *etc.* Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

The binding activity of a given lot of anti-HCAR or MCAR antibody may be determined according to well` known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation. Other such steps as washing, stirring,

shaking, filtering and the like may be added to the assays as is customary or necessary for the particular situation.

A preferred type of immunoassay to detect an antibody specific for a CAR protein according to the present invention is an enzyme-linked immunosorbent assay (ELISA) or more generally termed an enzyme immunoassay (EIA). In such assays, a detectable label bound to either an antibody-binding or antigen-binding reagent is an enzyme. This enzyme, in turn, when later exposed to its substrate, will react with the substrate in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric or by visual means. Enzymes which can be used to detectably label the reagents useful in the present invention include, but are not limited to, horseradish peroxidase, alkaline phosphatase, glucose oxidase, β-galactosidase, ribonuclease, urease, catalase, malate dehydrogenase, staphylococcal nuclease, asparaginase, Δ-5-steroid isomerase, yeast alcohol dehydrogenase, α-glycerophosphate dehydrogenase, triose phosphate isomerase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. For description of EIA procedures, see, for example, Voller, A. *et al.*, J. Clin. Pathol. 31:507-520 (1978); Butler, J.E., *Meth. Enzymol. 73*:482-523 (1981); Maggio, E. (ed.), *Enzyme Immunoassay*, CRC Press, Boca Raton, 1980; Butler, J.E., In: *STRUCTURE OF ANTIGENS*, Vol. 1 (Van Regenmortel, M., CRC Press, Boca Raton, 1992, pp. 209-259; Butler, J.E., In: van Oss, C.J. et al., (eds), *IMMUNOCHEMISTRY*, Marcel Dekker, Inc., New York, 1994, pp. 759-803; Butler, J.E. (ed.), *Immunochemistry of Solid-Phase Immunoassay*, CRC Press, Boca Raton, 1991)

In another embodiment, the detectable label may be a radiolabel, and the assay thus used termed a radioimmunoassay (RIA), which is well known in the art. See, for example, Yalow, R. *et al.*, Nature 184:1648 (1959); Work, T.S., *et al.*, *Laboratory Techniques and Biochemistry in Molecular Biology*, North Holland Publishing Company, NY, 1978, incorporated by reference herein. The radioactive isotope can be detected by such means as the use of a gamma counter

or a scintillation counter or by autoradiography. Isotopes which are particularly useful for the purpose of the present invention are ^{125}I, ^{131}I, ^{35}S, ^{3}H and ^{14}C.

It is also possible to label the antigen or antibody reagents with a fluorophore. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence of the fluorophore. Among the most commonly used fluorophores are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde, fluorescamine or fluorescence-emitting metals such as ^{152}Eu or other lanthanides. These metals are attached to antibodies using metal chelators.

The antigen or antibody reagents useful in the present invention also can be detectably labeled by coupling to a chemiluminescent compound. The presence of a chemiluminescent-tagged antibody or antigen is then determined by detecting the luminescence that arises during the course of a chemical reaction. Examples of useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester. Likewise, a bioluminescent compound such as a bioluminescent protein may be used to label the antigen or antibody reagent useful in the present invention. Binding is measured by detecting the luminescence. Useful bioluminescent compounds include luciferin, luciferase and aequorin.

Detection of the detectably labeled reagent according to the present invention may be accomplished by a scintillation counter, for example, if the detectable label is a radioactive gamma emitter, or by a fluorometer, for example, if the label is a fluorophore. In the case of an enzyme label, the detection can be accomplished by colorimetry which employs a substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

The immunoassay of this invention may be a "two-site" or "sandwich" assay. A first antibody specific for an epitope of the CAR protein is adsorbed to a solid support. After addition of the sample being tested for the presence of CAR

protein, , a quantity of detectably labeled soluble second antibody is added to permit detection and/or quantitation of the ternary complex formed between solid-phase antibody, antigen, and labeled antibody. Sandwich assays are generally described by Wide, *Radioimmune Assay Method*, Kirkham *et al.*, Eds., E. & S. Livingstone, Edinburgh, 1970, pp. 199-206.

According to the present invention, it is possible to diagnose circulating antibodies in a subject which are specific for the HCAR or MCAR protein. This is accomplished by means of an immunoassay, as described above, using the protein of the invention or a functional derivative thereof.

Based on similar principles, since a Ad2, Ad5 or CVB virus binds to its cellular receptor protein (HCAR or MCAR) with detectable affinity, it is possible to detect the presence of a Ad2, Ad5 or CVB virus capable of binding to HCAR or MCAR in a biological sample, using the HCAR or MCAR protein or a functional derivative thereof as a ligand. In such an assay, the protein or functional derivative may be adsorbed to an insoluble support or carrier, as in an immunoassay. The biological sample, *e.g.*, serum, suspected of containing Ad2, Ad5 or CVB virus is then contacted with the HCAR or MCAR-containing support and the virus allowed to bind to its receptor material. The presence of the bound virus is then revealed in any of a number of ways well known in the art, for example, by addition of a detectably-labeled antibody specific for the virus. The same assay can be used to detect the presence in a biological sample of a viral component such as a viral protein or glycoprotein which has affinity for the HCAR or MCAR protein. Alternatively, the virus or viral protein may be labeled and binding measured in a competitive assay using an antibody specific for the virus-binding portion of the HCAR or MCAR molecule.

As used herein, the term "prevention" of infection involves administration of the HCAR or MCAR protein, peptide derivative, or antibody (see above) prior to the clinical onset of the disease. Thus, for example, successful administration of a composition prior to initial contact with a Ad2, Ad5 or CVB virus results in

"prevention" of the disease. Administration may be after initial contact with the virus, but prior to actual development of the disease.

"Treatment" involves administration of the protective composition after the clinical onset of the disease. For example, successful administration of a HCAR or MCAR protein or peptide or anti-HCAR or MCAR antibody according to the invention after development of a Ad2, Ad5 or CVB virus infection in order to delay or suppress further virus spread comprises "treatment" of the disease.

The HCAR or MCAR protein, peptides or antibody of the present invention may be administered by any means that achieve their intended purpose, for example, to treat local infection or to treat systemic infection in a subject who has, or is susceptible to, such infection. For example, an immunosuppressed individual is particularly susceptible to viral infection and disease.

For example, administration may be by various parenteral routes such as subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, intracranial, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the oral route. Parenteral administration can be by bolus injection or by gradual perfusion over time.

An additional mode of using the compositions of the present invention is by topical application, primarily for disinfectant uses to minimize the risk of entry of Ad2, Ad5 or CVB viruses via the skin. The proteins, peptides and pharmaceutical compositions of the present invention may be incorporated into topically applied vehicles such as salves or ointments as a means for administering the active ingredient directly to the affected area. The carrier for the active ingredient may be either in sprayable or nonsprayable form. Non-sprayable forms can be semi-solid or solid forms comprising a carrier conducive to topical application and having a dynamic viscosity preferably greater than that of water. Suitable formulations include, but are not limited to, solution, suspensions, emulsions, creams, ointments, powders, liniments, salves, and the like. Preferred vehicles for non-sprayable topical preparations include ointment

bases, *e.g.*, polyethylene glycol-1000 (PEG-1000); conventional creams such as HEB cream; gels; as well as petroleum jelly and the like.

Also suitable for systemic or topical application, in particular to the mucus membranes and lungs, are sprayable aerosol preparations wherein the active ingredient, preferably in combination with a solid or liquid inert carrier material. The aerosol preparations can contain solvents, buffers, surfactants, perfumes, and/or antioxidants in addition to the proteins or peptides of the present invention. For aerosol administration, the active principles in accordance with the present invention may be packaged in a squeeze bottle, or in a pressurized container with an appropriate system of valves and actuators. Preferably, metered valves are used with the valve chamber being recharged between actuation or dose, all as is well known in the art.

For topical applications, it is preferred to administer an effective amount of a compound according to the present invention to an infected area, *e.g.*, skin surfaces, mucous membranes, *etc.* This amount will generally range from about 0.001 mg to about 1 g per application, depending upon the area to be treated, whether the use is prophylactic or therapeutic, the severity of the symptoms, and the nature of the topical vehicle employed. A preferred topical preparation is an ointment wherein about 0.01 to about 50 mg of active ingredient is used per cc of ointment base, the latter being preferably PEG-1000.

A typical regimen for preventing, suppressing, or treating Ad2, Ad5 or CVB virus infection comprises administration of an effective amount of the HCAR or MCAR protein or functional derivative thereof, administered over a period of one or several days, up to and including between one week and about six months.

It is understood that the dosage administered *in vivo* or *in vitro* will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The ranges of effective doses provided below are not intended to be

limiting and represent preferred dose ranges. However, the most preferred dosage will be tailored to the individual subject, as is understood and determinable by one of skill in the art.

The total dose required for each treatment may be administered by multiple doses or in a single dose. The protein, functional derivative thereof or antibody may be administered alone or in conjunction with other therapeutics directed to the viral infection, or directed to other symptoms of the viral disease.

Effective amounts of the HCAR or MCAR protein, functional derivative thereof, or antibody thereto, are from about 0.01 μg to about 100 mg/kg body weight, and preferably from about 10 μg to about 50 mg/kg body weight.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions, which may contain auxiliary agents or excipients which are known in the art. Pharmaceutical compositions such as tablets and capsules can also be prepared according to routine methods.

Pharmaceutical compositions comprising the proteins, peptides or antibodies of the invention include all compositions wherein the protein, peptide or antibody is contained in an amount effective to achieve its intended purpose. In addition, the pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically.

Pharmaceutical compositions include suitable solutions for administration by injection or orally, and contain from about 0.01 to 99%, preferably from about 20 to 75% of active component (*i.e.*, the HCAR or MCAR protein, peptide or antibody) together with an excipient. Pharmaceutical compositions for oral administration include tablets and capsules. Compositions which can be administered rectally include suppositories.

The present invention provides methods for evaluating the presence and the level of normal or mutant HCAR or MCAR protein or mRNA in a subject. Absence, or more typically, low expression of the HCAR or MCAR gene or presence of a mutant HCAR or MCAR in an individual may serve as a predictor of resistance to Ad2, Ad5 or CVB virus infection. Alternatively, over-expression of HCAR or MCAR, may serve as a predictor of enhanced susceptibility to Ad2, Ad5 or CVB virus infection.

Oligonucleotide probes encoding any of a number of segments of the HCAR or MCAR DNA sequence are used to test cells from a subject for the presence HCAR or MCAR DNA or mRNA. A preferred probe would be one directed to the nucleic acid sequence encoding at least 12 and preferably at least 15 nucleotides of the HCAR or MCAR sequence. Qualitative or quantitative assays can be performed using such probes. For example, Northern analysis (see below) is used to measure expression of an HCAR or MCAR mRNA in a cell or tissue preparation.

Such methods can be used even with very small amounts of DNA obtained from an individual, following use of selective amplification techniques. Recombinant DNA methodologies capable of amplifying purified nucleic acid fragments are well-known and typically involve introduction of the nucleic acid fragment into a DNA or RNA vector, the clonal amplification of the vector, and the recovery of the amplified nucleic acid fragment (U.S. Patent 4,237,224; Sambrook *et al.* (*supra*))

Preferably a method based on polymerase chain reaction (PCR) is used to selectively increase the concentration of a particular nucleic acid sequence even when that sequence has not been previously purified and is present only in a single copy in a particular sample. The method can be used to amplify either single- or double-stranded DNA. The oligonucleotide sequences of the probes of the PCR method are selected such that they contain sequences identical to, or complementary to, sequences which flank the particular nucleic acid sequence

whose amplification is desired. More specifically, the oligonucleotide sequences of the "first" probe is selected such that it is capable of hybridizing to an oligonucleotide sequence located 3' to the desired sequence, whereas the oligonucleotide sequence of the "second" probe is selected such that it contains an oligonucleotide sequence identical to one present 5' to the desired region. Both probes possess 3' hydroxy groups which permits primer extension. The PCR reaction is capable of exponential amplification of specific nucleic acid sequences because the extension product of the "first" probe, of necessity, contains a sequence which is complementary to a sequence of the "second" probe, and thus can serve as a template for the production of an extension product of the "second" probe. Similarly, the extension product of the "second" probe, of necessity, contains a sequence which is complementary to a sequence of the "first" probe, and thus can serve as a template for the production of an extension product of the "first" probe. Thus, by permitting cycles of polymerization, and denaturation, a geometric increase in the concentration of the desired nucleic acid molecule can be achieved. Reviews of the PCR are provided by Mullis, K.B. (*Cold Spring Harbor Symp. Quant. Biol. 51*:263-273 (1986)); Saiki, R.K., *et al.* (*Bio/Technology 3*:1008-1012 (1985)); and Mullis, K.B., *et al.* (*Meth. Enzymol. 155*:335-350 (1987); see also: Erlich H. *et al.*, EP 50,424; EP 84,796, EP 258,017, EP 237,362; Mullis, K., EP 201,184; Mullis K. *et al.*, US 4,683,202; Erlich, H., US 4,582,788; and Saiki, R. *et al.*, US 4,683,194)).

Oligonucleotide probes corresponding to a portion of the HCAR or MCAR DNA sequence are useful in the new method of reverse transcriptase-PCR ("RT-PCR"). Isolated mRNA is reverse-transcribed into DNA, and then the DNA fragments corresponding to HCAR or MCAR are specifically amplified by PCR. This technique has wide application in biology and medicine The approach can be used in solution or *in situ.* RT-PCR permits comparison the patterns of expression of HCAR or MCAR with another gene. RT-PCR for the detection of circulating tumor cells is as a potential technique for detecting or staging cancer involving HCAR or MCAR-expressing cells. For reviews, see, for example,

Larrick, J.W., *Trends. Biotechnol.*,1992, *10*:146-152; Teo, I.A. *et al., Histochem. J.*, 1995, 27:647-659; Schultz, G.A. *et al., Reprod. Fertil. Dev.* 1992, *4*:361-371; Pelkey, T.J. *et al., Clin. Chem.*, 1996, *42*:1369-1381).

Having now generally described the invention, the same will be more readily understood through reference to the following example which is provided by way of illustration, and is not intended to be limiting of the present invention, unless specified.

EXAMPLE I

Materials and Methods for the following Examples

cDNA Library Screening

Five μg of TCMK-1 RNA selected twice with oligo (dT) was used to construct a cDNA library in the λ ZAP Express® vector using the ZAP Express cDNA synthesis kit (Stratagene). A library of $4x10^5$ primary clones was amplified once in the XL1-Blue MRF' strain of *E. coli*. The library was plated at a density of $5x10^4$ PFU on 150mm diameter NZY agar plates and overlaid with nitrocellulose filters that had been wet in sterile dH_20 containing 10 mM IPTG (isopropylthio-β-D-galactoside) after incubation for 2.5 hours at 42°C. The plates were transferred to 37°C for 8 hours before the filters were removed and washed once in Tris buffered saline to remove agarose and bacteria. Thereafter, filters were assayed for the presence of the expressed protein product with anti-p46 antiserum (Xu, R. *et al., supra*) using the BCIP/NBT detection system.

Northern Blots

Analysis of cellular RNA was conducted by transfer to nylon membranes following electrophoretic separation through a 1.2% formaldehyde agarose gel according to published procedures (Davis, L.G. *et al.*, (Elsevier Science Publishing Co. New York, NY 1986)). Hybridizations were performed in 50% formamide, 1% SDS and 5X SSPE for 18-24 hours at 42°C. Membranes were

washed once in 2X SSPE, 0.1% SDS at room temperature, and twice in 0.1X SSPE, 0.1% SDS at 42°C for 30 minutes following hybridization.

Transfections

$3x10^5$ NIH3T3 cells were seeded into 35mm plates 24 hours prior to transfection. Forty- five minutes prior to transfection, a solution was prepared by mixing 2μg of supercoiled plasmid diluted in 100μl of DMEM (Gibco) and 7μl of lipofectamine (Life Technologies, GIBCO) diluted in 100μl of DMEM. Following incubation at room temperature, 800μl of DMEM was added and the solution overlaid onto plates that were washed twice with DMEM. The plates were incubated for 5 hours at 37°C in 5% CO_2 before the solution was removed and 2 ml of DMEM containing 10% fetal calf serum (FCS) was added. The plates were incubated for an additional 18 hours at 37°C. Cells were used 18-24 hours post-transfection for experiments.

Virus Assays

Twenty hours following transfection, 35mm plates containing $5x10^5$ cells were washed twice, and 200μl DMEM containing $1x10^7$ pfu of virus were added. Plates were incubated for 90 minutes at room temperature before being washed 4 times with 2ml of DMEM. Plates were overlaid with 5ml DMEM/10% FCS and twenty-four hours later alternately frozen and thawed three times. Cellular debris was removed by centrifugation, and dilutions of the resulting supernatants evaluated for virus content by plaque assay (Crowell, R.L. *et al., J. Exp. Med. 113*:419-435 (1961)).

Western Blots

Cells were solubilized in 1% Triton X-100, 1% deoxycholate, and analyzed by 10% SDS-PAGE using the discontinuous buffer system (Laemmli, U.K. *Nature 227*:680-685 (1970)). Following transfer to PVDF membrane supports, blots were probed with either 125ng/ml of the mAb RmcB or a 1:1000 dilution of anti-p46 antiserum for one hour at room temperature.

Immunoreactivity was determined following incubation using horseradish peroxidase conjugated secondary antibodies and the ECL Western Blotting Detection System (Amersham).

Immunofluorescence

$1x10^6$ transfectant cells were incubated in 100 µl of 1X PBS containing 2µg of RmcB for 1 hour at 4°C. Cells were washed three times with 1X PBS and incubated for 30 minutes at 4°C in 100 µl of 1X PBS containing 0.35µg of a goat anti-mouse FITC conjugated antibody. Fluorescence was monitored using a Zeiss Axioplan fluorescent microscope.

EXAMPLE II

Cloning of HCAR and MCAR Molecules

To isolate a receptor molecule, the present inventors employed the classical approach of screening a λ phage expression cDNA library with an antiserum containing antibodies specific for a mouse CVB binding protein. The protein is termed p46 and the antibodies, anti-p46 (Xu, R. *et al., Vir. Res. 35*:323-340 (1995)). This antibody has several properties that indicate its specificity for the viral receptors sought. First, it protects mouse cells from infection with CVB. Second, the antibody specifically identified a 46 kDa protein from virus-susceptible mouse cells (Xu, R. *et al., Vir. Res. 35*:323-340 (1995)).

From a transformed mouse kidney cell (TCMK-1) library, a 1 kb cDNA clone (RTMCAR-4) was isolated that hybridized in Northern blots with (6kb and 1.4kb RNA's from TCMK-1 cells that were not found in receptor-negative mouse L cells (Hsu K-H. L. *et al., J. Virol. 63*:3105-8 (1989)) (Figure 1). "Blast" searches of the Genbank EST database using the nucleotide sequence of RTMCAR-4 revealed two IMAGE consortium clones from newborn human melanocyte (265680) and pancreas islet cell (328668) cDNA libraries that were

significantly homologous to RTMCAR-4. These findings suggested the human clones may encode a homologue of RTMCAR-4.

To proceed, clone 265680 was purchased from the American Type Culture Collection and the nucleotide and deduced amino acid sequence compared to that of RTMCAR-4. Based upon best fit alignments, both 265680 and RTMCAR-4 contained single open reading frames that exhibited 67% amino acid identity. Clone 265680 was slightly larger, containing an additional 34 amino acids N-terminal of the protein encoded by RTMCAR-4.

However, neither clone appeared to contain the entire coding sequence, since their open reading frames (ORFs), which lacking starting methionine codons, began immediately at the 5' end of each clone. Therefore, primers for amplification of the 5' ends were designed specifically for either RTMCAR-4 or clone 265680 and used in the polymerase chain reaction (PCR) to amplify fragments of 450 base-pairs (bp) and 280 bp from TCMK-1 and HeLa cell cDNA libraries, respectively.

These sequences were sufficiently long to include the starting ATG codon and to encode the additional amino acids necessary to complete the open reading frames.

These DNA molecules and their encoded proteins have the following properties shown in Table 2.:

TABLE 2

Virus Receptor	cDNA size (approx)	Number of amino acid residues encoded	Molecular mass of protein (approx.)
MCAR	1.4 kb	352	39 kDa
HCAR	2.4 kb	365	40 kDa

Although the coding regions of the two cDNA's are similar, the human clone contains 1.2 kb of 3' untranslated sequence.

These molecules are 83% identical with conservative amino acid (aa) substitutions found at most of the differing positions (Figure 2A and 2B). The only dramatic difference between the molecules is a sequence divergence at the carboxyl terminus. Both proteins contain potential leader sequences, transmembrane domains, and two N-linked glycosylation sites. Structurally they contain two disulfide bonded loops (aa 35-130 and aa 155-220), which resemble the characteristic V and C2 domains of the Ig superfamily (Williams, A.F. *et al.*, *Ann. Rev. Immun.* *6*:381-405 (1988)). However, no significant homologies have been found during "Blast" searches between sequences present in the SWISSPROT database and HCAR or MCAR.

EXAMPLE III

HCAR and MCAR are Functional Viral Receptors

To determine if the HCAR and MCAR cDNA's encode functional receptors, they were inserted into pBK-CMV vectors, transfected into NIH3T3 cells. The plasmid pRTHR contains a 1.1kb HCAR cDNA that lacks the 3'UTR. The plasmid pRTMR contains the complete 1.4kb MCAR cDNA. Transfectants were assayed for virus production or reporter gene expression..

The transfected cells, upon viral infection, yielded 10^2- to 10^3-fold higher titers of infectious Coxsackie virus, CVB3 and CVB4, than did cells transfected with control plasmid (pBK-CMV) alone. The infectability was specific for CVB, since no increase in titer was seen when another picornavirus family member, poliovirus T1, was used (Table 3).

In addition, pRTHR and pRTMR, but not plasmid control transfectants, stained positively for (β-galactosidase 16 hours after incubation with a recombinant Ad2 expressing βgal from the CMV promoter (Figure 3).

TABLE 3

Production of Plaque Forming Units (PFU) following Virus Infection of Transfected Cells

	INFECTING VIRUS		
Transfecting Plasmid	*CVB3*	*CVB4*	*Poliovirus*
pRTHR	$2.92x10^7$	$1.7x10^6$	$2.65x10^4$
pRTMR	$4.22x10^6$	$6.0x10^5$	$2.60x10^4$
pBK-CMV	$2.25x10^4$	$1.5x10^3$	$2.27x10^4$

HCAR and MCAR are specific adenovirus binding proteins, since ^{35}S-Ad3 (a group B adenovirus), which does not compete with group C adenoviruses for the same receptor (Defer, C. *et al., J. Virol. 64*:3661-3673 (1990)), exhibits 5-fold less binding to transfectants than does ^{35}S-Ad2. The binding of labeled virus could be competed with cold virus, as previously described (Lonberg-Holm *et al., supra*). Thus, these results confirm that HCAR and MCAR are the receptors for Ad2, A5, and the CVB.

EXAMPLE IV

Immunological Detection of HCAR and MCAR Proteins on Transfected Cells

The ability of the viruses to infect NIH3T3 cells indicated that the receptors were being properly expressed in this cell type. Immunofluorescence using the well defined monoclonal antibody (mAb) specific for human CVB receptor (designated RmcB), which can block infection in human cells of CVB viruses (Hsu, K-H. L *et al., J. Virol. 62*:1647-1652 (1988)), revealed a bright staining of the surface of HCAR transfectants. Photomicrographs revealed that HCAR expression was localized to the exterior of the cell. Bright staining was observed over the entire plasma membrane in unfixed NIH3T3 cells transfected with pRTHR and labeled with RmcB and FITC labeled goat anti-mouse antibody Controls pBK-CMV transformed control cells showed no staining. These results

confirmed that receptors were localized on the external side of the plasma membrane.

Western blots of detergent solubilized cell extracts detected two proteins of about 46kDa and 44kDa from HCAR transfectants and HeLa cell positive controls (although much less for the 44kDa protein). In contrast, the pBK-CMV (control) transfectants did not produce any signal (central lane of Figure 4A and 4B. When the rat anti-p46 (Xu, R. *et al., Vir. Res. 35*:323-340 (1995)) antiserum was used to probe MCAR transfectant extracts, a very strong signal was also obtained at an apparent molecular mass of 46kDa.

In both cases, the translated receptor proteins appear to have undergone posttranslational processing which increased their size from about 40kDa to about 46kDa. The doublet observed with HCAR may be a result of glycosylation of the 46 kDa molecule at both of its N-linked sites, whereas the 44kDa molecules may only be glycosylated at a single site. The HCAR-specific mAb did not react with the MCAR product, and the anti-MCAR serum did not react with the HCAR product, as previously reported (Crowell, R.L. *et al.*, In: *Cellular Receptors for Animal Viruses,* Wimmer, E., ed., Cold Spring Harbor Press, New York, 1994, pp. 75-79 .

EXAMPLE V

Expression of HCAR and MCAR RNA in Transfected Cells

Northern blots of poly A-selected RNA probed with fragments from the coding regions of HCAR and MCAR identified RNA's of about 6kb and about 1.4 kb from TCMK-1 cells, and a range of RNA's from about 6kb to about 1.3 kb from HeLa cells (Figure 5).

Neither fragment hybridized to RNA's of the respective receptor-negative cells of human (rhabdomyosarcoma) or murine (L cell) origin.

The larger mRNA's may be incompletely processed transcripts or RNA's that are related to the receptors by sequence homology. Nonetheless, the sizes of the identified cDNA's correlate with sizes of RNA's detected by these hybridizations (Figure 5), indicating they are truly representative of the parental RNA species.

PolyA-selected RNA's from multiple human tissues or mouse tissues were probed with HCAR probes or MCAR probes, respectively, in Northern blots. The pancreas, brain, heart, small intestine, testis, and prostate had the highest amount of HCAR message (Figure 6). The liver and lung had small amounts of HCAR mRNA. No signal was detected in kidney, placenta, peripheral blood leukocytes, thymus, and spleen. In contrast, in mouse tissue, the highest level of MCAR RNA was observed in liver (Figure 7). Mouse kidney, heart, lung and brain tissue also expressed detectable levels of MCAR RNA. Importantly, the expression of HCAR at the RNA level appears to correlate well with the organ pattern of infectability by CVB (Melnick, J.L., In: *Virology* (Fields B.N. *et al.*, eds.) Raven Press, New York, 1990, pp. 549-600)

Isolation of the genomic sequences for both HCAR and MCAR will provide insight into the regulation of their expression which is useful information for identifying possible targets for adenovirus gene therapy or devising strategies to inhibit adenovirus and CVB infectivity.

EXAMPLE VI

A Structural Model for the CAR Proteins and their Interaction with CBV3 and the Ad5 Fiber Knob

The hydrophobicity profile for the HCAR and MCAR sequences revealed two strongly hydrophobic stretches: fourteen N-terminal residues, a hypothetical signal peptide, and residues 239-259, a possible transmembrane helix. A sensitive sequence search (Abagyan, R.A., *et al. Submitted to J. Mol. Biol.* (1996)) of fragment 15-235 through the Swissprot database and the NCBI non-redundant database revealed a number of similarities with membrane-bound proteins, most of them containing Ig-like domains. The protein sequences shown in Table 4, below, contain the strongest similarities to the extracellular portion of the CAR (the Swissprot code or only its first part is given in parentheses):

Weaker similarities were found with T-cell surface glycoprotein CD4 and B lymphocyte activation antigen CD86. Although the level of sequence identity for all the proteins listed above was below the safe confidence level of about 30% , the scores of the Needleman and Wunsch alignments (Needleman S.B., *et al. J. Mol. Biol., 48*:443-453 (1970)) were highly significant ($P < 0.000001$) according to the inventors' statistics (Abagyan, R.A., *et al. Submitted to J. Mol. Biol.* (1996)). Additionally, two cysteines (C41 and C120), which can form a disulfide bridge in the immunoglobulin fold, were found at characteristic positions.

TABLE 4

PROTEIN	Swissprot Code
butyrophilin precursor	BUTY
basement membrane-specific heparan sulfate proteoglycan core	PGBM
fibroblast growth factor receptor BFR-2	BFR2
fasciclin II	FS21, FS22, FAS2
vascular endothelial growth factor receptor 1,)	VEGR, VGR1
basement membrane proteoglycan	UN52_CAEEL
T lymphocyte activation antigen CD80	
axon-associated cell adhesion molecule	AXO1
myelin P0 protein	MYP0
neuroglial precursor	NRG_DROME
neural cell adhesion molecule L1	CAML_RAT
contactin	CONT_CHICK
connectin 3B	PIR: pn0568
basic fibroblast growth factor receptor 1	FGR4_HUMAN
B-lymphocyte activation antigen 7 from Mouse	GBL12585
vascular cell adhesion protein 1	VCA1_HUMAN
amalgam protein	AMAL_DROME

Model building

We built a model of the first Ig-like domain (IG1) of the extracellular part of the HCAR and MCAR on the basis of presumed sequence and structural similarity to the monoclonal antibody against cholera toxin peptide 3 (PDB code 1tet). It has a sequence identity of 28% over about 100 residues. The second Ig-like domain, IG2, and the connection between IG1 and IG2 (residues 127-233) were built by homology with the residues 89 to 178 of the T-cell surface glycoprotein CD4 (PDB code 3cd4). The sequence identity between the CD4 fragment and the 127-233 region of the receptor was 21%. The sequence of IG1 and IG2 domains was threaded onto 1tet and 3cd4; the initial sequence alignment

was adjusted to make it consistent with the immunoglobulin topology. The side-chains were placed by global energy minimization (Abagyan, R.A., *et al. J.Comp.Chem., 15*:488-506 (1994)).

Docking of the receptor

Docking of the 3D model of the CVB3 receptor to the canyon of the virus was performed on the basis of the low resolution structure of the human rhinovirus with the ICAM1 receptor, as well as shape complementarity between the receptor model and the structure of the CVB3 (1cox). Analytical molecular surface was built with the contour-buildup algorithm (Totrov, M.M., *et al. J. Struct. Biol., 116*:138-143 (1996)) implemented in the ICM program. Docking solutions have been generated by the Brownian Monte-Carlo procedure (Abagyan, R.A., *et al. J.Comp.Chem., 15*:488-506 (1994); Totrov, M.M., *et al. Nature Structural Biology, 1*:259-263 (1994)).

RESULTS

Direct comparison of the extracellular fragment of the two CARs with the sequences of proteins with known 3D structure showed heavy chains of FAB fragments of immunoglobulins [protein data base (pdb) codes 1tet, 1iai, 1dbb, 1ggi, 1mco, 7fab, *etc.*] at the top of the hit list. The heavy chain of anticholera toxin (1tet) aligns with 27% sequence identity. From the alignments, it was concluded that two Ig-like domains, further referred to as IG1 and IG2, form the extracellular part of the receptor.

The cytoplasmic domain was found similar to a number of so called Ser-Pro-Thr-rich domains, which are found in a TFG gene related to the papillary thyroid carcinoma and p68 TRK-T3 oncoprotein. No related proteins with known function or 3D structure were found. In the beginning of the cytoplasmic domain there is a fragment RKKRREEKY (residues 265-273 in SEQ ID NO:2 and SEQ ID NO:4), matching two possible signatures for a tyrosine kinase phosphorylation site: ([RK]-x-x-[DE]-x-x-x-Y, or [RK]-x-x-x-[DE]-x-x-Y).

Tyrosine 269, therefore, may be involved in signal transduction via tyrosine phosphorylation and dephosphorylation. Three other tyrosines do not have the required upstream sequence pattern, and are less likely, although still possible, candidates for signal transduction.

To build a 3D model of the IG1 domain we used homology to the heavy chain of an antibody (1tet) with known 3D structure. The rest of the extracellular domain was built by homology with CD4 (3cd4). We used the ICM program (Molsoft) to thread the receptor sequence onto its 3D template and to "energy minimize" the model.

Receptor interaction with the Coxsackie virus:

The CBV3 structure was solved to 3.5 Å resolution recently (Muckelbauer, J.K., *et al. Acta Crystallorg., Sect. D., 51:*871-887 (1995)). These investigators predicted that the primary receptor-binding site on CVB3 is the canyon which is formed around the 5-fold axis of icosahedral picornaviruses. Cryoelectron microscopy of another picornavirus, a human rhinovirus, with its receptor, ICAM-1 (Olson, N.H., *et al. Proc. Natl. Acad. Sci. USA, 90:*507-511 (1993)), showed that the monomeric N-terminal Ig-like domain D1 of ICAM-1 contains the primary binding site which interacts with the canyon of HRV16. Two Ig-like amino-terminal domains of CD4 receptor of the HIV-1 for which the structure has been determined at atomic resolution (Wang, J.H., *et al. Nature, 348:*411-418 (1990); Ryu, S.E., *et al. Nature, 348:*419-426 (1990)) were used instead of the ICAM-1 subunit to reconstruct the HRV14-ICAM-1 complex at 28 Å resolution (Olson, N.H., *et al. Proc. Natl. Acad. Sci. USA, 90:*507-511 (1993)) and confirm the proposed binding of the tip of the first Ig-like domain of the receptor to the canyon.

We analyzed the molecular surface and electrostatic potential of the canyon in the 3.5 A resolution structure of CVB3 (Figures 8-11) and performed docking of the HCAR model to the canyon. The tip of IG1 domain of the HCAR model fits into the canyon and, therefore, we can hypothesize that it fits to the

canyon similarly to how the D1 domain of ICAM-1 fits into the canyon of a rhinovirus.

Receptor interaction with the adenovirus fiber knob: Xia et al. (Xia, D., *et al. Structure, 2*:1259-1270 (1994)) solved the structure of the carboxy-terminal knob domain of the Ad5 fiber at 1.7 Å resolution and suggested that both the central surface depression surrounding the three-fold axis (Figure 9) and the side valleys are conserved and are candidates for binding sites of the cellular receptor.

We analyzed the surface of the trimer and concluded that the central surface depression around the three-fold axis is not a favorable binding site for the IG1 domain of the receptor. In contrast, the IG1 domain fits well to the side-valley of the trimer. It forms an extended interaction interface in such a way that two β sheets of the knob and two β sheets of the receptor form a sandwich of four sheets. According to the proposed model (Figure 9), one, two, or three receptor molecules can fit simultaneously with the knob without steric clashes.

EXAMPLE VI

A. Mutation analysis to map virus binding sites of HCAR

A mutation analysis of of linear HCAR amino acid sequences was conducted to determine the sites critical for AdC or CVB binding. Standard PCR methods have been and are being usesd. The table below summarizes some of the results obtained using amino acid substitutions within regions proposed above to be critical for either Ad C or CVB binding:

Virus	Mutation From	To	EFFECT
CVB	**DLKSG**	**DAEEG**	None
AdC	**QVIIL**	**QAIDL**	Inhibition of Binding
AdC and CVB	**PEDQG**	**PADQG**	None

B. Identification of genomic sequences encoding HCAR

Studies were undertaken to to identify the promoter sequences that regulate HCAR expression, and to determine the relationship between a 7kb transcript observed in Northern blots of HCAR expressing cells and the functional 2.5kb cDNA message. Several EcoRI restriction fragments have been cloned from a yeast artificial chromosome (YAC) that contains a portion of human chromosome 21 DNA. These sequences hybridize with the HCAR cDNA as reported above. (Genebank accession U90716). Isolation of the promoter region will reveal information that will allow *in vitro* and *in vivo* manipulation of receptor expression by small molecules acting on factors that are part of the HCAR transcription complex.

C. Analysis of cDNA molecules encoding alternate carboxyl termini of MCAR.

The 3' ends of MCAR transcripts were analyzed by reverse transcriptase PCR (RT-PCR). Total RNA extracted from various tissues of a 3 week old female Balb/C mouse was reverse transcribed, and the resulting cDNA PCR was amplified using a 3'- anchor primer and a 5'-primer specific for MCAR. Several amplified products were obtained and subjected to DNA sequencing. Three distinct sequences were identified that give rise to alternate receptor carboxyl termini. Two of the sequences appear to be present in all tissues analyzed. The third has been isolated only from heart. The amino acid sequence of these alternate forms is likely to be conserved from residues 1- 339 of SEQ ID NO:2 or SEQ ID NO:4. Following lysine residue 339, the sequences deviate among the alternate forms, presumably through alternate splicing.

The references cited above are all incorporated by reference herein, whether specifically incorporated or not.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: CELLULAR RECEPTORS FOR SUBGROUP C ADENOVIRUSES AND GROUP B COXSACKIEVIRUSES

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
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(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2354 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1095

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ATG GCG CTC CTG CTG TGC TTC GTG CTC CTG TGC GGA GTA GTG GAT TTC        48
Met Ala Leu Leu Leu Cys Phe Val Leu Leu Cys Gly Val Val Asp Phe
 1               5                  10                  15
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GCC AGA AGT TTG AGT ATC ACT ACT CCT GAA GAG ATG ATT GAA AAA GCC          96
Ala Arg Ser Leu Ser Ile Thr Thr Pro Glu Glu Met Ile Glu Lys Ala
             20                  25                  30

AAA GGG GAA ACT GCC TAT CTG CCA TGC AAA TTT ACG CTT AGT CCC GAA         144
Lys Gly Glu Thr Ala Tyr Leu Pro Cys Lys Phe Thr Leu Ser Pro Glu
         35                  40                  45

GAC CAG GGA CCG CTG GAC ATC GAG TGG CTG ATA TCA CCA GCT GAT AAT         192
Asp Gln Gly Pro Leu Asp Ile Glu Trp Leu Ile Ser Pro Ala Asp Asn
     50                  55                  60

CAG AAG GTG GAT CAA GTG ATT ATT TTA TAT TCT GGA GAC AAA ATT TAT         240
Gln Lys Val Asp Gln Val Ile Ile Leu Tyr Ser Gly Asp Lys Ile Tyr
 65                  70                  75                  80

GAT GAC TAC TAT CCA GAT CTG AAA GGC CGA GTA CAT TTT ACG AGT AAT         288
Asp Asp Tyr Tyr Pro Asp Leu Lys Gly Arg Val His Phe Thr Ser Asn
                 85                  90                  95

GAT CTC AAA TCT GGT GAT GCA TCA ATA AAT GTA ACG AAT TTA CAA CTG         336
Asp Leu Lys Ser Gly Asp Ala Ser Ile Asn Val Thr Asn Leu Gln Leu
            100                 105                 110

TCA GAT ATT GGC ACA TAT CAG TGC AAA GTG AAA AAA GCT CCT GGT GTT         384
Ser Asp Ile Gly Thr Tyr Gln Cys Lys Val Lys Lys Ala Pro Gly Val
        115                 120                 125

GCA AAT AAG AAG ATT CAT CTG GTA GTT CTT GTT AAG CCT TCA GGT GCG         432
Ala Asn Lys Lys Ile His Leu Val Val Leu Val Lys Pro Ser Gly Ala
    130                 135                 140

AGA TGT TAC GTT GAT GGA TCT GAA GAA ATT GGA AGT GAC TTT AAG ATA         480
Arg Cys Tyr Val Asp Gly Ser Glu Glu Ile Gly Ser Asp Phe Lys Ile
145                 150                 155                 160

AAA TGT GAA CCA AAA GAA GGT TCA CTT CCA TTA CAG TAT GAG TGG CAA         528
Lys Cys Glu Pro Lys Glu Gly Ser Leu Pro Leu Gln Tyr Glu Trp Gln
                165                 170                 175

AAA TTG TCT GAC TCA CAG AAA ATG CCC ACT TCA TGG TTA GCA GAA ATG         576
Lys Leu Ser Asp Ser Gln Lys Met Pro Thr Ser Trp Leu Ala Glu Met
            180                 185                 190

ACT TCA TCT GTT ATA TCT GTA AAA AAT GCC TCT TCT GAG TAC TCT GGG         624
Thr Ser Ser Val Ile Ser Val Lys Asn Ala Ser Ser Glu Tyr Ser Gly
        195                 200                 205

ACA TAC AGC TGT ACA GTC AGA AAC AGA GTG GGC TCT GAT CAG TGC CTG         672
Thr Tyr Ser Cys Thr Val Arg Asn Arg Val Gly Ser Asp Gln Cys Leu
    210                 215                 220

TTG CGT CTA AAC GTT GTC CCT CCT TCA AAT AAA GCT GGA CTA ATT GCA         720
Leu Arg Leu Asn Val Val Pro Pro Ser Asn Lys Ala Gly Leu Ile Ala
225                 230                 235                 240

GGA GCC ATT ATA GGA ACT TTG CTT GCT CTA GCG CTC ATT GGT CTT ATC         768
Gly Ala Ile Ile Gly Thr Leu Leu Ala Leu Ala Leu Ile Gly Leu Ile
                245                 250                 255
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ATC TTT TGC TGT CGT AAA AAG CGC AGA GAA GAA AAA TAT GAA AAG GAA        816
Ile Phe Cys Cys Arg Lys Lys Arg Arg Glu Glu Lys Tyr Glu Lys Glu
            260                 265                 270

GTT CAT CAC GAT ATC AGG GAA GAT GTG CCG CCT CCA AAG AGC CGT ACG        864
Val His His Asp Ile Arg Glu Asp Val Pro Pro Pro Lys Ser Arg Thr
        275                 280                 285

TCC ACT GCC AGA AGC TAC ATC GGC AGT AAT CAT TCA TCC CTG GGG TCC        912
Ser Thr Ala Arg Ser Tyr Ile Gly Ser Asn His Ser Ser Leu Gly Ser
    290                 295                 300

ATG TCT CCT TCC AAC ATG GAA GGA TAT TCC AAG ACT CAG TAT AAC CAA        960
Met Ser Pro Ser Asn Met Glu Gly Tyr Ser Lys Thr Gln Tyr Asn Gln
305                 310                 315                 320

GTA CCA AGT GAA GAC TTT GAA CGC ACT CCT CAG AGT CCG ACT CTC CCA       1008
Val Pro Ser Glu Asp Phe Glu Arg Thr Pro Gln Ser Pro Thr Leu Pro
                325                 330                 335

CCT GCT AAG GTA GCT GCC CCT AAT CTA AGT CGA ATG GGT GCG ATT CCT       1056
Pro Ala Lys Val Ala Ala Pro Asn Leu Ser Arg Met Gly Ala Ile Pro
            340                 345                 350

GTG ATG ATT CCA GCA CAG AGC AAG GAT GGG TCT ATA GTA TAGAGCCTCC        1105
Val Met Ile Pro Ala Gln Ser Lys Asp Gly Ser Ile Val
        355                 360                 365

ATATGTCTCA TCTGTGCTCT CCGTGTTCCT TTCCTTTTTT TGATATATGA AAACCTATTC     1165

TGTTCTAAAT TGTGTTACTA GCCTCAAAAT ACATCAAAAA ATAAGTTAAT CAGGAACTGT     1225

ACGGAATATA TTTTTAAAAA TTTTTGTTTG GTTATATCGA AATAGTTACA GGCACTAAAG     1285

TTAGTAAAGA AAAGTTTACC ATCTGAAAAA GCTGGATTTT CTTTAAGAGG TTGATTATAA     1345

AGGTTTCTAA ATTTATCAGT ACCTAAGTAA GATGTAGCGC TTTGAATATG AAATCATAGG     1405

TGAAGACATG GGTGAACTTA CTTGCATACC AAGTTGATAC TTGAATAACC ATCTGAAAGT     1465

GGTACTTGAT CATTTTTACC ATTATTTTTA GGATGTGTAT TTCATTTATT TATGGCCCAC     1525

CAGTCTCCCC CAAATTAGTA CAGAAATATC CATGACAAAA TTACTTACGT ATGTTTGTAC     1585

TTGGTTTTAC AGCTCCTTTG AAAACTCTGT GTTTGGAATA TCTCTAAAAA CATAGAAAAC     1645

ACTACAGTGG TTTAGAAATT ACTAATTTTA CTTCTAAGTC ATTCATAAAC CTTGTCTATG     1705

AAATGACTTC TTAAATATTT AGTTGATAGA CTGCTACAGG TAATAGGGAC TTAGCAAGCC     1765

CTTTTATATG CTAAAGGAGC ATCTATCAGA TTAAGTTAGA ACATTTGCTG TCAGCCACAT     1825

ATTGAGATGA CACTAGGTGC AATAGCAGGG ATAGATTTTG TTGGTGAGTA GTCTCATGCC     1885

TTGAGATCTG TGGTGGTCTT CAAAATGGTG GCCAGCCAGA TCAAGGATGT AGTATCTCAT     1945

AGTTCCCCAG GTGATATTTT TCTTATTAGA AAAATATTAT AACTCATTTG TTGTTTGACA     2005

CTTATAGATT GAAATTTCCT AATTTATTCT AAATTTTAAG TGGTTTCTTT GGTTCCAGTG     2065

CTTTATGTTG TTGTTGTTTT TGGATGGTGT TACATATTAT ATGTTGTAGA AACATGTAAT     2125
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CCTAAATTTA CCCTCTTGAA TATAATCCCT GGATGATATT TTTTATAATA AATGCAGAAT 2185

AATCAAATAC ATTTTAAGCA AGTTAAGTGT CCTCCATCAA TTCTGTATTC CAGACTTGGG 2245

AGGATGTACA GTTGCTGTTG TGTGATCAAA CATGTCTCTG TGTAGTTCCA GCAAATCAAG 2305

CTGAGCTTTG AAAAAGTTTG TCTTAGTTTT GTGAAGGTGA TTTATTCTT 2354

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 365 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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            20                  25                  30

Lys Gly Glu Thr Ala Tyr Leu Pro Cys Lys Phe Thr Leu Ser Pro Glu
        35                  40                  45

Asp Gln Gly Pro Leu Asp Ile Glu Trp Leu Ile Ser Pro Ala Asp Asn
    50                  55                  60

Gln Lys Val Asp Gln Val Ile Ile Leu Tyr Ser Gly Asp Lys Ile Tyr
65                  70                  75                  80

Asp Asp Tyr Tyr Pro Asp Leu Lys Gly Arg Val His Phe Thr Ser Asn
                85                  90                  95

Asp Leu Lys Ser Gly Asp Ala Ser Ile Asn Val Thr Asn Leu Gln Leu
            100                 105                 110

Ser Asp Ile Gly Thr Tyr Gln Cys Lys Val Lys Lys Ala Pro Gly Val
        115                 120                 125

Ala Asn Lys Lys Ile His Leu Val Val Leu Val Lys Pro Ser Gly Ala
    130                 135                 140

Arg Cys Tyr Val Asp Gly Ser Glu Glu Ile Gly Ser Asp Phe Lys Ile
145                 150                 155                 160

Lys Cys Glu Pro Lys Glu Gly Ser Leu Pro Leu Gln Tyr Glu Trp Gln
                165                 170                 175

Lys Leu Ser Asp Ser Gln Lys Met Pro Thr Ser Trp Leu Ala Glu Met
            180                 185                 190

Thr Ser Ser Val Ile Ser Val Lys Asn Ala Ser Ser Glu Tyr Ser Gly
        195                 200                 205

Thr Tyr Ser Cys Thr Val Arg Asn Arg Val Gly Ser Asp Gln Cys Leu
    210                 215                 220
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Leu Arg Leu Asn Val Val Pro Pro Ser Asn Lys Ala Gly Leu Ile Ala
225                 230                 235                 240

Gly Ala Ile Ile Gly Thr Leu Leu Ala Leu Ala Leu Ile Gly Leu Ile
                245                 250                 255

Ile Phe Cys Cys Arg Lys Lys Arg Arg Glu Glu Lys Tyr Glu Lys Glu
            260                 265                 270

Val His His Asp Ile Arg Glu Asp Val Pro Pro Pro Lys Ser Arg Thr
        275                 280                 285

Ser Thr Ala Arg Ser Tyr Ile Gly Ser Asn His Ser Ser Leu Gly Ser
    290                 295                 300

Met Ser Pro Ser Asn Met Glu Gly Tyr Ser Lys Thr Gln Tyr Asn Gln
305                 310                 315                 320

Val Pro Ser Glu Asp Phe Glu Arg Thr Pro Gln Ser Pro Thr Leu Pro
                325                 330                 335

Pro Ala Lys Val Ala Ala Pro Asn Leu Ser Arg Met Gly Ala Ile Pro
            340                 345                 350

Val Met Ile Pro Ala Gln Ser Lys Asp Gly Ser Ile Val
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```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1301 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 157..1212

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GTTTACCTGC AAGCCACGAC CCGGCCGGCA GCTACC ATG GCG CGC CTA CTG TGC        174
                                        Met Ala Arg Leu Leu Cys
                                                                5

TTC GTG CTC TTG TGC GGG ATC GCG GAT TTC ACC AGT GGT TTG AGC ATC        222
Phe Val Leu Leu Cys Gly Ile Ala Asp Phe Thr Ser Gly Leu Ser Ile
            10                  15                  20

ACT ACA CCC GAA CAG AGG ATC GAA AAA GCC AAA GGG GAA ACT GCG TAT       270
Thr Thr Pro Glu Gln Arg Ile Glu Lys Ala Lys Gly Glu Thr Ala Tyr
        25                  30                  35
```

```
CTA CCA TGC AAG TTT ACT CTC AGT CCC GAA GAC CAG GGA CCA CTG GAC        318
Leu Pro Cys Lys Phe Thr Leu Ser Pro Glu Asp Gln Gly Pro Leu Asp
     40                  45                  50

ATT GAA TGG CTG ATA TCC CCG TCT GAT AAC CAG ATA GTG GAT CAG GTG        366
Ile Glu Trp Leu Ile Ser Pro Ser Asp Asn Gln Ile Val Asp Gln Val
 55                  60                  65                  70

ATC ATT TTG TAT TCT GGA GAC AAA ATT TAT GAT AAC TAC TAT CCG GAT        414
Ile Ile Leu Tyr Ser Gly Asp Lys Ile Tyr Asp Asn Tyr Tyr Pro Asp
                 75                  80                  85

CTG AAA GGA CGG GTA CAT TTT ACG AGT AAC GAT GTC AAG TCT GGC GAC        462
Leu Lys Gly Arg Val His Phe Thr Ser Asn Asp Val Lys Ser Gly Asp
             90                  95                 100

GCA TCT ATA AAT GTG ACC AAC CTG CAG CTG TCG GAC ATT GGC ACT TAC        510
Ala Ser Ile Asn Val Thr Asn Leu Gln Leu Ser Asp Ile Gly Thr Tyr
        105                 110                 115

CAG TGC AAA GTG AAG AAA GCC CCT GGG GTT GCA AAT AAG AAA TTC CTG        558
Gln Cys Lys Val Lys Lys Ala Pro Gly Val Ala Asn Lys Lys Phe Leu
    120                 125                 130

CTG ACC GTT CTT GTT AAG CCT TCA GGT ACA AGA TGC TTC GTG GAT GGA        606
Leu Thr Val Leu Val Lys Pro Ser Gly Thr Arg Cys Phe Val Asp Gly
135                 140                 145                 150

TCG GAA GAG ATT GGA AAT GAC TTC AAG CTA AAA TGT GAA CCC AAG GAA        654
Ser Glu Glu Ile Gly Asn Asp Phe Lys Leu Lys Cys Glu Pro Lys Glu
                155                 160                 165

GGC TCC CTT CCA CTA CAG TTT GAA TGG CAG AAA CTG TCG GAC TCC CAG        702
Gly Ser Leu Pro Leu Gln Phe Glu Trp Gln Lys Leu Ser Asp Ser Gln
            170                 175                 180

ACA ATG CCT ACG CCA TGG CTG GCA GAA ATG ACG TCA CCA GTT ATA TCT        750
Thr Met Pro Thr Pro Trp Leu Ala Glu Met Thr Ser Pro Val Ile Ser
        185                 190                 195

GTG AAG AAC GCC AGT TCT GAG TAT TCT GGG ACA TAC AGC TGC ACG GTT        798
Val Lys Asn Ala Ser Ser Glu Tyr Ser Gly Thr Tyr Ser Cys Thr Val
    200                 205                 210

CAA AAC AGA GTG GGC TCT GAC CAG TGT ATG CTG CGA CTA GAC GTT GTC        846
Gln Asn Arg Val Gly Ser Asp Gln Cys Met Leu Arg Leu Asp Val Val
215                 220                 225                 230

CCA CCC TCC AAC CGA GCC GGA ACG ATC GCG GGC GCC GTC ATA GGG ACG        894
Pro Pro Ser Asn Arg Ala Gly Thr Ile Ala Gly Ala Val Ile Gly Thr
                235                 240                 245

CTG CTG GCC CTT GTG CTC ATC GGG GCC ATC CTC TTC TGC TGT CAC AGG        942
Leu Leu Ala Leu Val Leu Ile Gly Ala Ile Leu Phe Cys Cys His Arg
            250                 255                 260

AAA CGC AGA GAA GAG AAG TAC GAG AAG GAA GTT CAT CAT GAT ATC AGG        990
Lys Arg Arg Glu Glu Lys Tyr Glu Lys Glu Val His His Asp Ile Arg
        265                 270                 275
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Glu Asp Val Pro Pro Pro Lys Ser Arg Thr Ser Thr Ala Arg Ser Tyr
    280                 285                 290

ATT GGC AGC AAC CAT TCC TCC CTG GGA TCC ATG TCC CCC TCT AAC ATG      1086
Ile Gly Ser Asn His Ser Ser Leu Gly Ser Met Ser Pro Ser Asn Met
295                 300                 305                 310

GAG GGG TAT TCC AAG ACG CAG TAT AAC CAA GTC CCC AGT GAA GAC TTT      1134
Glu Gly Tyr Ser Lys Thr Gln Tyr Asn Gln Val Pro Ser Glu Asp Phe
                315                 320                 325

GAA CGT GCG CCT CAG AGC CCG ACT TTG GCA CCC GCT AAG TTC AAG TAC      1182
Glu Arg Ala Pro Gln Ser Pro Thr Leu Ala Pro Ala Lys Phe Lys Tyr
            330                 335                 340

GCT TAC AAG ACC GAT GGC ATT ACA GTG GTA TAAATATGAA GGACTACTGA       1232
Ala Tyr Lys Thr Asp Gly Ile Thr Val Val
        345                 350

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GTTTTTTGG                                                          1301
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 352 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ala Arg Leu Leu Cys Phe Val Leu Leu Cys Gly Ile Ala Asp Phe
  1               5                  10                  15

Thr Ser Gly Leu Ser Ile Thr Thr Pro Glu Gln Arg Ile Glu Lys Ala
            20                  25                  30

Lys Gly Glu Thr Ala Tyr Leu Pro Cys Lys Phe Thr Leu Ser Pro Glu
        35                  40                  45

Asp Gln Gly Pro Leu Asp Ile Glu Trp Leu Ile Ser Pro Ser Asp Asn
    50                  55                  60

Gln Ile Val Asp Gln Val Ile Ile Leu Tyr Ser Gly Asp Lys Ile Tyr
65                  70                  75                  80

Asp Asn Tyr Tyr Pro Asp Leu Lys Gly Arg Val His Phe Thr Ser Asn
                85                  90                  95

Asp Val Lys Ser Gly Asp Ala Ser Ile Asn Val Thr Asn Leu Gln Leu
            100                 105                 110

Ser Asp Ile Gly Thr Tyr Gln Cys Lys Val Lys Lys Ala Pro Gly Val
        115                 120                 125

Ala Asn Lys Lys Phe Leu Leu Thr Val Leu Val Lys Pro Ser Gly Thr
    130                 135                 140
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Arg Cys Phe Val Asp Gly Ser Glu Glu Ile Gly Asn Asp Phe Lys Leu
145                 150                 155                 160

Lys Cys Glu Pro Lys Glu Gly Ser Leu Pro Leu Gln Phe Glu Trp Gln
                165                 170                 175

Lys Leu Ser Asp Ser Gln Thr Met Pro Thr Pro Trp Leu Ala Glu Met
            180                 185                 190

Thr Ser Pro Val Ile Ser Val Lys Asn Ala Ser Ser Glu Tyr Ser Gly
        195                 200                 205

Thr Tyr Ser Cys Thr Val Gln Asn Arg Val Gly Ser Asp Gln Cys Met
    210                 215                 220

Leu Arg Leu Asp Val Val Pro Pro Ser Asn Arg Ala Gly Thr Ile Ala
225                 230                 235                 240

Gly Ala Val Ile Gly Thr Leu Leu Ala Leu Val Leu Ile Gly Ala Ile
                245                 250                 255

Leu Phe Cys Cys His Arg Lys Arg Arg Glu Glu Lys Tyr Glu Lys Glu
            260                 265                 270

Val His His Asp Ile Arg Glu Asp Val Pro Pro Pro Lys Ser Arg Thr
        275                 280                 285

Ser Thr Ala Arg Ser Tyr Ile Gly Ser Asn His Ser Ser Leu Gly Ser
    290                 295                 300

Met Ser Pro Ser Asn Met Glu Gly Tyr Ser Lys Thr Gln Tyr Asn Gln
305                 310                 315                 320

Val Pro Ser Glu Asp Phe Glu Arg Ala Pro Gln Ser Pro Thr Leu Ala
                325                 330                 335

Pro Ala Lys Phe Lys Tyr Ala Tyr Lys Thr Asp Gly Ile Thr Val Val
            340                 345                 350
```

WHAT IS CLAIMED IS:

1. An isolated DNA molecule or an allelic variant thereof which

(a) encodes a human receptor protein or glycoprotein molecule termed HCAR or a murine receptor protein or glycoprotein termed MCAR which protein or glycoprotein is a receptor for coxsackievirus B, Ad2 and Ad5, or

(b) encodes a functional derivative thereof,

wherein said protein, glycoprotein or functional derivative binds to group C adenoviruses or coxsackievirus B.

2. A DNA molecule according to claim 1 having the coding sequence of SEQ ID NO:1 or SEQ ID NO:3.

3. A DNA molecule according to claim 1 wherein said protein has the amino acid sequence SEQ ID NO:2 or SEQ ID NO:4.

4. A DNA molecule according to claim 1 wherein said functional derivative is a peptide of an extracellular immunoglobulin domain of HCAR or MCAR.

5. A DNA molecule according to claim 4 wherein said peptide is selected from the group consisting of

(a) residues 35-130 of SEQ ID NO:2 or of SEQ ID NO:4; and

(b) residues 155-220 of SEQ ID NO:2 or of SEQ ID NO:4.

6. A DNA molecule according to claim 4 wherein said peptide comprises:

an amino acid sequence selected from the group consisting of LSPEDQGP, PEDQG, LDIEW, QVIIL, QMIIL, ILYSGD, DKII, NDLKS, NDVKS and VKKAPG, or

between one and about twenty repeats of an oligopeptide sequence selected from the group consisting of (a) LSPEDQGP, (b) PEDQG, (c) LDIEW, (d) QVIIL, (e) QMIIL, (f) ILYSGD, (g) DKII, (h) NDLKS, (I) NDVKS, (j) VKKAPG or a combination of any of (a) - (j).

7. A DNA molecule according to claims 1 which is an expression vector.

8. A prokaryotic host transformed with the DNA molecule of claim 7

9. A eukaryotic host cell transformed or transfected with a DNA molecule according to claim 7.

10. A process for preparing a HCAR or MCAR protein, glycoprotein or functional derivative molecule which, if naturally occurring, is substantially free of other proteins or glycoproteins with which it is natively associated, comprising:

(a) culturing a host cell transformed with the expression vector of claim 7 under culturing conditions to express said DNA molecule; and

(c) recovering said protein, glycoprotein or functional derivative molecule from said culture.

11. A HCAR or MCAR protein, glycoprotein or derivative made according to the process of claim 10.

12. A HCAR or MCAR protein, glycoprotein or functional derivative molecule which, if naturally occurring, is substantially free of other proteins or glycoproteins with which it is natively associated, which protein, glycoprotein or functional derivative is encoded by the DNA molecule of claim 1.

13. A HCAR or MCAR protein, glycoprotein or functional derivative molecule which, if naturally occurring, is substantially free of other proteins or glycoproteins with which it is natively associated, which protein, glycoprotein or functional derivative is encoded by the DNA molecule of claim 2.

14. A HCAR or MCAR functional derivative according to claim 12 which is a peptide of an extracellular immunoglobulin domain of said HCAR or MCAR.

15. A peptide according to claim 14 which is selected from the group consisting of

(a) residues 35-130 of SEQ ID NO:2 or of SEQ ID NO:4; and

(b) residues 155-220 of SEQ ID NO:2 of of SEQ ID NO:4.

16. A functional derivative according to claim 12 which is a peptide comprising:

an amino acid sequence selected from the group consisting of (a) LSPEDQGP, (b) PEDQG, (c) LDIEW, (d) QVIIL, (e) QMIIL, (f) ILYSGD, (g) DKII, (h) NDLKS, (i) NDVKS, (j) VKKAPG, (k) a combination of any of (a) - (j), or

between one and about twenty repeats of an oligopeptide sequence selected from the group consisting of (a) LSPEDQGP, (b) PEDQG, (c) LDIEW, (d) QVIIL, (e) QMIIL, (f) ILYSGD, (g) DKII, (h) NDLKS, (i) NDVKS, (j) VKKAPG, (k) a combination of any of (a) - (j).

17. A composition comprising a HCAR or MCAR protein, glycoprotein or functional derivative according to claim 12, immobilized to a solid support.

18. A method for detecting the presence or measuring the quantity of HCAR or MCAR protein, glycoprotein or functional derivative in a biological sample, comprising:

(a) contacting said biological sample that is suspected of containing said protein, glycoprotein or derivative with a binding partner capable of binding to said HCAR or MCAR protein; and

(b) detecting the binding of said binding partner to a substance in said sample or measuring the quantity of said binding partner bound,

thereby determining the presence or measuring the quantity of said HCAR or MCAR protein, glycoprotein or derivative.

19. The method of claim 18, wherein said binding partner is an antibody or an antigen-binding fragment thereof.

20. The method of claim 18, wherein said binding partner is a viral protein or peptide which binds to said HCAR or MCAR protein or is an HCAR or MCAR-binding functional derivative of said viral protein or peptide.

21. A method for identifying in a sample an analyte capable of binding to a HCAR or MCAR protein, glycoprotein or functional derivative according to claims 12, which method comprises:

(a) incubating said sample which is suspected of containing said analyte in the presence of an HCAR or MCAR protein, glycoprotein or functional derivative, or a ligand-binding portion thereof, such that said HCAR or MCAR protein, glycoprotein, derivative or portion binds to said analyte; and

(b) detecting said analyte which is bound to said protein, glycoprotein, derivative or potion,

thereby detecting the presence of said analyte in said sample.

22. A method according to claim 21 wherein said HCAR or MCAR protein, glycoprotein, derivative or portion is immobilized to a solid support.

23. A method according to claim 21 wherein said analyte is a virus, a viral protein or peptide, or an HCAR- or MCAR-specific antibody.

24. A method for isolating from a complex mixture a composition capable of binding to HCAR or MCAR protein, glycoprotein or functional derivative of claim 12, comprising:

(a) immobilizing said HCAR or MCAR protein, glycoprotein or derivative, or a ligand-binding portion thereof, to a solid support;

(b) contacting said complex mixture with said solid support of step (a) so that any of said composition binds to said immobilized HCAR or MCAR protein, glycoprotein or derivative;

(c) washing away any unbound material from said mixture; and

(d) eluting said bound composition,

thereby isolating said composition.

25. A pharmaceutical composition useful for preventing or treating an infection by a virus which utilizes HCAR or MCAR as its cellular receptor, comprising:

(a) a protein, glycoprotein or functional derivative according to claim 12; and

(b) a pharmaceutically acceptable carrier or excipient.

26. A method for preventing or treating in a subject an infection with a virus which utilizes HCAR or MCAR as its cellular receptor, comprising administering to said subject an effective amount of a pharmaceutical composition according to claim 25.

27. A method for inhibiting the infectivity of adenovirus serotype 2 or serotype 5, or of a group B coxsackievirus, comprising contacting said virus with an effective amount of the protein, glycoprotein or derivative molecule according to claim 12 and allowing said molecule to prevent said virus from attaching to a cell, thereby inhibiting said infectivity

28. A method for rendering a cell normally not susceptible to infection with adenovirus susceptible to infection by, and virus-mediated gene transfer by, an adenovirus vector, comprising the steps of:

(a) transforming a cell of said non-susceptible species or cell type with an expression vector according to claim 7;

(b) expressing said HCAR or MCAR protein, glycoprotein or derivative on the surface of said cell

thereby rendering said cell susceptible to infection by said adenoviral vector.

29. A method for transferring a gene with an adenovirus vector to a cell of an animal species or a cell type normally resistant to adenovirus-mediated gene transfer, comprising:

(a) culturing a cell intended to receive said transferred gene;

(b) rendering said cell susceptible by the method of claim 28; and

(c) infecting said cell with said adenovirus vector carrying the gene to be transferred

thereby transferring said gene.

Fig. 1

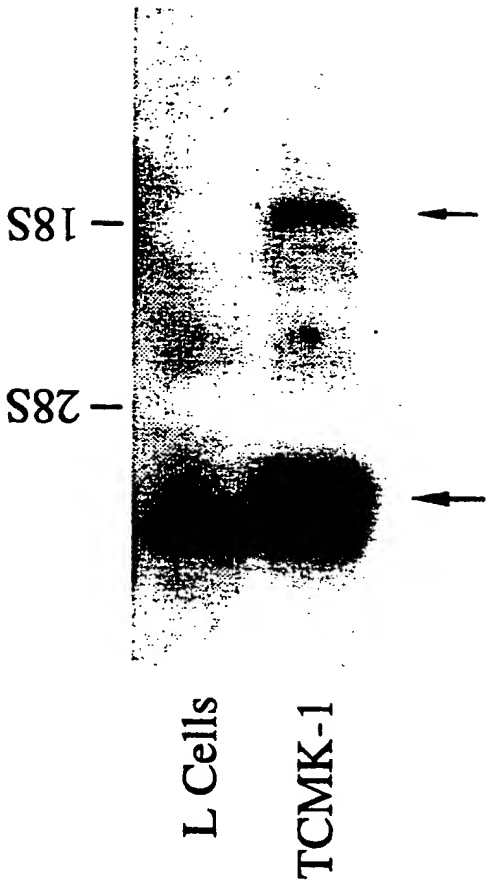


Fig. 2A

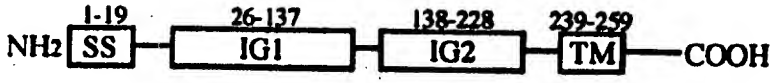
```
MCAR   MARLLCFVLL CGIADFTSGL SITTPEQRIE KAKGETAYLP CKFTLSPEDQ GPLDIEWLIS PSDNQIVDQV IILYSGDKIY DNIYPDLKGR VHFTSNDVKS
HCAR   MALLLCFVLL CGVVDFARSL SITTPEEMIE KAKGETAYLP CKFTLSPEDQ GPLDIEWLIS PADNQKVDQV IILYSGDKIY DDIYPDLKGR VHFTSNDLKS

MCAR   GDASINVTNL QLSDIGTYQC KVKKAPGVAN KKFLLTVLVK PSGTRCFVDG SEEIGNDFKL KCEPKEGSLP LQFEWQKLSD SQTMPTPWLA EMTSPVISVK
HCAR   GDASINVTNL QLSDIGTYQC KVKKAPGVAN KKIHLVVLVK PSGARCYVDG SEEIGSDFKI KCEPKEGSLP LQYEWQKLSD SQKMPTSWLA EMTSSVISVK

MCAR   NASSEYSGTY SCTVQNRVGS DQCMLRLDVV PPSNRAGTIA GAVIGTLLAL VLIGAILFCC HRKRREEKYE KEVHHDIRED VPPPKSRTST ARSYIGSNHS
HCAR   NASSEYSGTY SCTVRNRVGS DQCLLRLNVV PPSNKAGLIA GAIIGTLLAL ALIGLIIFCC RKKRREEKYE KEVHHDIRED VPPPKSRTST ARSYIGSNHS

MCAR   SLGSMSPSNM EGYSKTQYNQ VPSEDFERAP QSPTLAPAKF KYAY--KTDG I--------- --TVV                                    352
HCAR   SLGSMSPSNM EGYSKTQYNQ VPSEDFERTP QSPTLPPAKV AAPNLSRMGA IPVMIPAQSK DGSIV                                    365
```

Fig. 2B



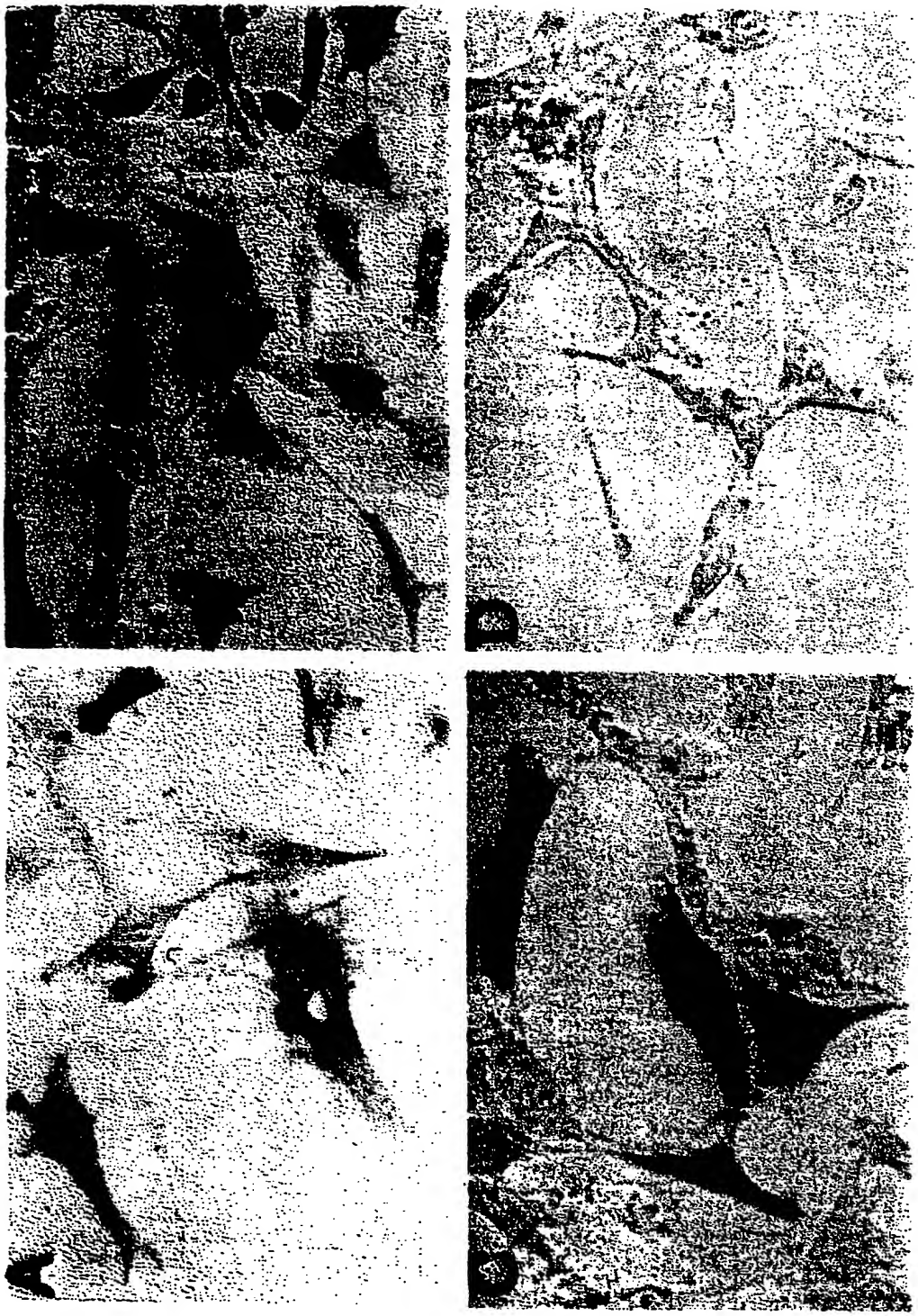
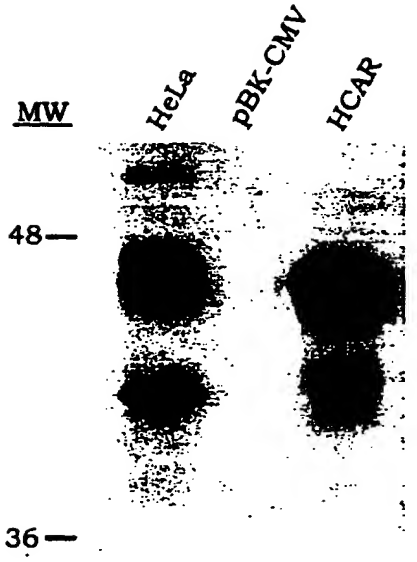
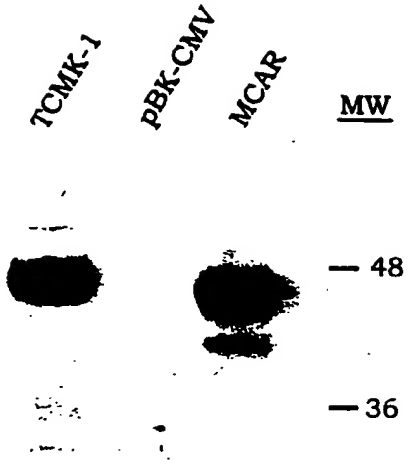


Fig. 3



RmcB

Fig. 4A



anti-p46

Fig. 4B

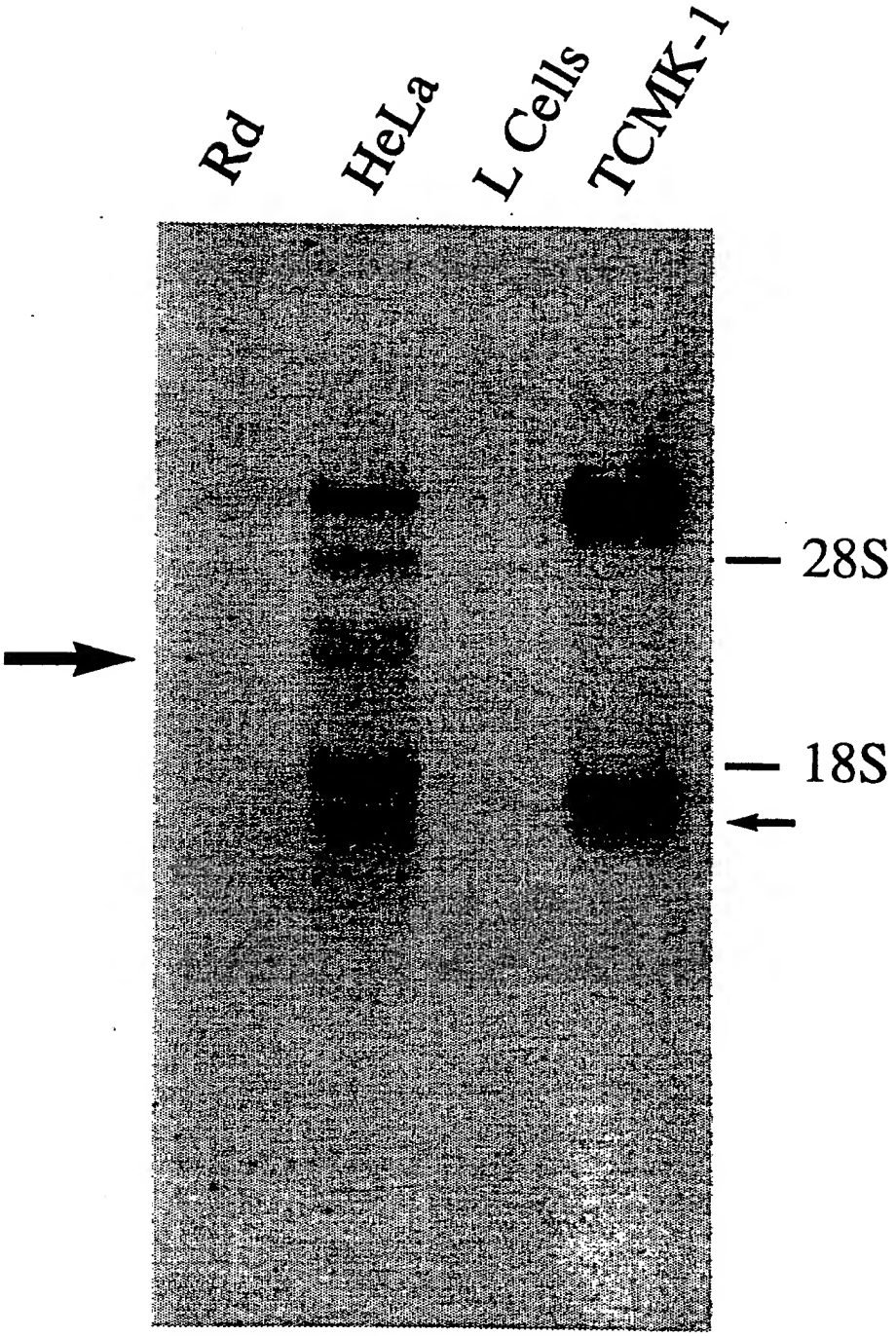


Fig. 5

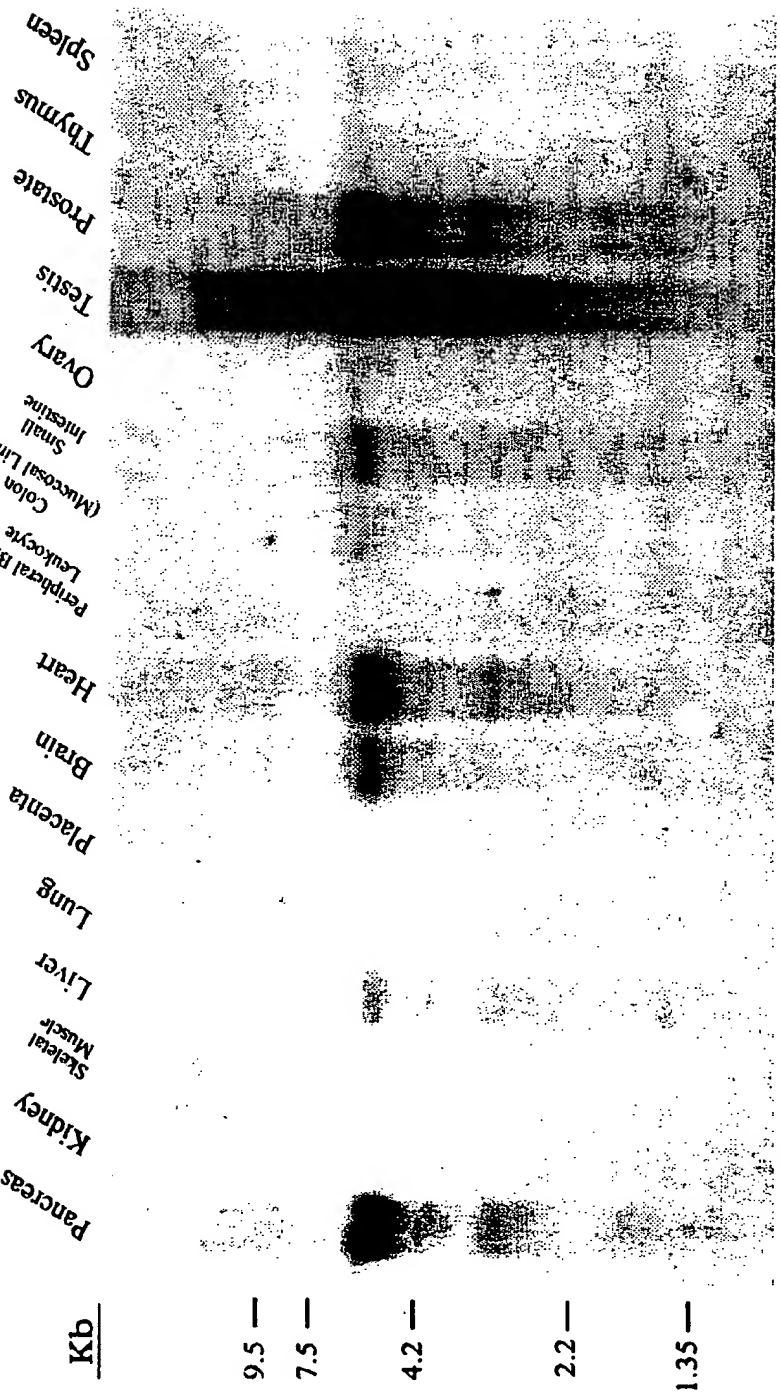

Kb
9.5
7.5
4.2
2.2
1.35
Pancreas
Kidney
Skeletal Muscle
Liver
Lung
Placenta
Brain
Heart
Peripheral Blood Leukocyte
Colon (Mucosal Lining)
Small Intestine
Ovary
Testis
Prostate
Thymus
Spleen

Fig. 6

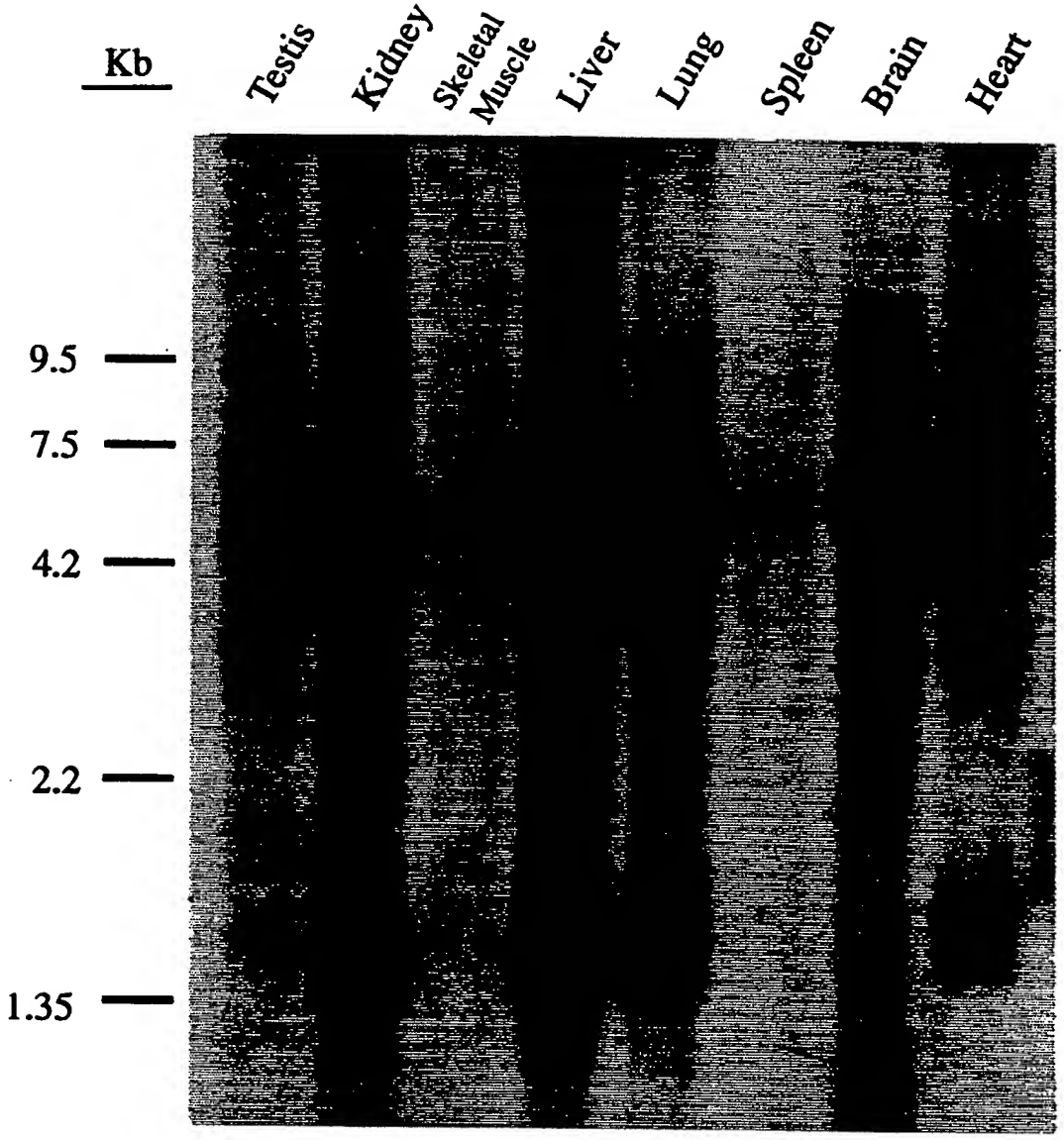

Kb
Testis
Kidney
Skeletal Muscle
Liver
Lung
Spleen
Brain
Heart
9.5
7.5
4.2
2.2
1.35

Fig. 7

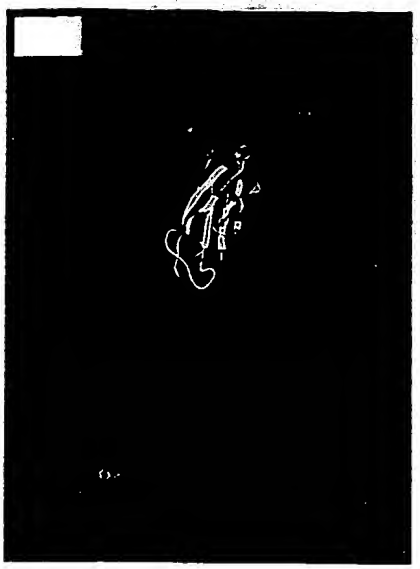


Fig. 8

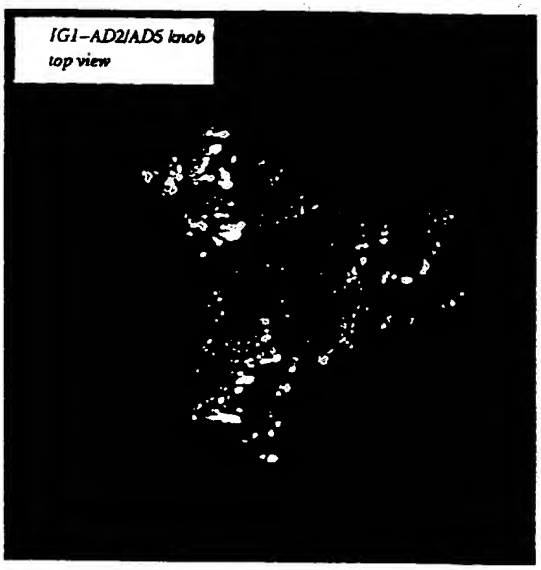


Fig. 9

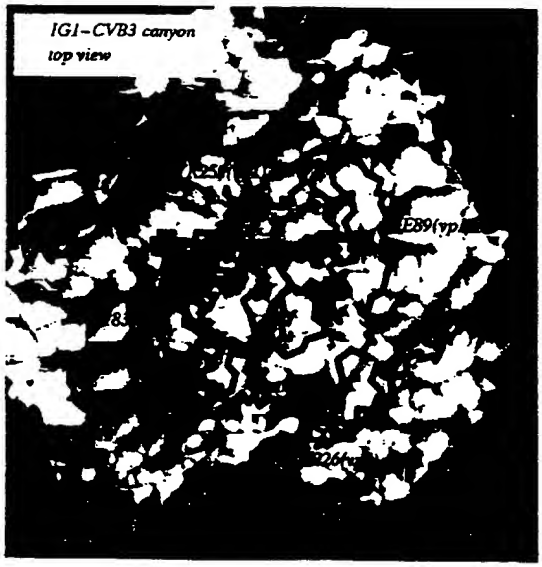


Fig. 10

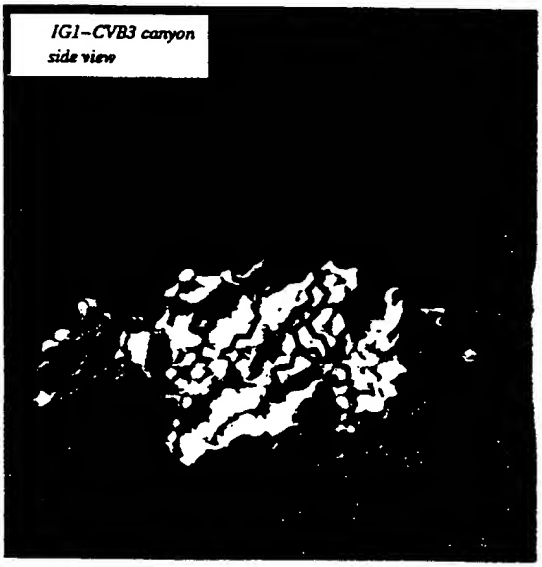


Fig. 11

Fig. 12

HCAR Amino Acid Sequence (SEQ ID NO:2)

```
                                                        *****
MALLLCFVLL CGVVDFARSL SITTPEEMIE KAKGETAYLP CKFTLSPEDQ GPLDIEWLIS   60
                                                     ^^^^^^ ^^

         ** ***   ***
PADNQKVDQV IILYSGDKIY DDYYPDLKGR VHFTSNDLKS GDASINVTNL QLSDIGTYQC  120
           ^^^^^^                      ^^^^^

KVKKAPGVAN KKIHLVVLVK PSGARCYVDG SEEIGSDFKI KCEPKEGSL PLQYEWQKLS  180
DSQKMPTSWL AEMTSSVISV KNASSEYSGT YSCTVRNRVG SDQCLLRLNV VPPSNKAGLI  240
AGAIIGTLLA LALIGLIIFC CRKKRREEKY EKEVHHDIRE DVPPPKSRTS TARSYIGSNH  300
SSLGSMSPSN MEGYSKTQYN QVPSEDFERT PQSPTLPPAK VAAPNLSRMG AIPVMIPAQS  360
KDGSIV                                                             365
```

MCAR Amino Acid Sequence (SEQ ID NO:4)

```
                                                        *****
MARLLCFVLL CGIADFTSGL SITTPEQRIE KAKGETAYLP CKFTLSPEDQ GPLDIEWLIS   60
                                                     ^^^^^^ ^^

         ** ***   ***
PSDNQIVDQV IILYSGDKIY DNYYPDLKGR VHFTSNDVKS GDASINVTNL QLSDIGTYQC  120
           ^^^^^^                      ^^^^^

KVKKAPGVAN KKFLLTVLVK PSGTRCFVDG SEEIGNDFKL KCEPKEGSLP LQFEWQKLSD  180
SQTMPTPWLA EMTSPVISVK NASSEYSGTY SCTVQNRVGS DQCMLRLDVV PPSNRAGTIA  240
GAVIGTLLAL VLIGAILFCC HRKRREEKYE KEVHHDIRED VPPPKSRTST ARSYIGSNHS  300
SLGSMSPSNME GYSKTQYNQ VPSEDFERAP QSPTLAPAKF KYAYKTDGIT VV          352
```

KEY TO ANNOTATION:

- Ad2/Ad5-binding regions:..... * above line
- CVB-binding regions: ..... ^ below line
- Ad2/5-and CVB-binding regions:.. underscore

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01724

A. CLASSIFICATI N OF SUBJECT MATTER
IPC(6) :C07K 14/705; C12N 15/12; G01N 33/566
US CL :435/7.1, 69.1; 436/501; 530/350; 536/23.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1, 69.1; 436/501; 530/350; 536/23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN/MEDLINE
search terms: adenovir?, coxsackievir?, receptor#

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P —— Y,P	BERGELSON et al. Isolation of a Common Receptor for Coxsackie B Viruses and Adenoviruses 2 and 5. Science. 28 February 1997, Vol. 275, pages 1320-1323, see entire document.	1-23 —— 24-29
X,P —— Y,P	TOMKO et al. HCAR and MCAR: The human and mouse cellular receptors for subgroup C adenoviruses and group B coxsackieviruses. Proceedings of the National Academy of Science. April 1997, Vol. 94, pages 3352-3356, see entire document.	1-23, 28, 29 ——24-27
X —— Y	XU et al. Receptor proteins on newborn Balb/c mouse brain cells for coxsackievirus B3 are immunologically distinct from those on HeLa cells. Virus Research. 1995, Vol. 35, pages 323-340, see in particular Figure 1.	11, 12, 16-23 —— 1-10, 13-15, 24-29

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 01 APRIL 1998	Date of mailing of the international search report 26 MAY 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JOHN D. ULM Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01724

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SEED et al. Molecular cloning of the CD2 antigene, the T-cell erythrocyte receptor, by a rapid immunoselection procedure. Proceedings of the National Academy of Science. May 1987, Vol. 84, pages 3365-3369, see entire document.	1-10, 13-15, 24-29

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01724

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SEED et al. Molecular cloning of the CD2 antigene, the T-cell erythrocyte receptor, by a rapid immunoselection procedure. Proceedings of the National Academy of Science. May 1987, Vol. 84, pages 3365-3369, see entire document.	1-10, 13-15, 24-29